Diethylene glycol

Health-based recommended occupational exposure limit

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Minister van Sociale Zaken en Werkgelegenheid

onderwerp : Aanbieding advies *Diethylene glycol*
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Geachte minister,

Graag bied ik u hierbij het advies aan over de beroepsmatige blootstelling aan diethyleenglycol. Het maakt deel uit van een uitgebreide reeks, waarin gezondheidskundige adieswaarden worden afgeleid voor concentraties van stoffen op de werkplek. Dit advies over diethyleenglycol is opgesteld door de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen (GBBS) van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

Ik heb dit advies vandaag ter kennisname toegezonden aan de minister van Volksgezondheid, Welzijn en Sport, de minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer en de staatssecretaris van Sociale Zaken en Werkgelegenheid.

Hoogachtend,

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Diethylene glycol

Health-based recommended occupational exposure limit

Dutch Expert Committee on Occupational Standards
a committee of the Health Council of the Netherlands

to:

the Minister of Health, Welfare and Sport

No. 2007/03OSH, The Hague, 17 October 2007

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Samenvatting

Vraagstelling

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid leidt de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen (GBBS, voorheen WGD) van de Gezondheidsraad gezondheidskundige advieswaarden af voor stoffen in de lucht op de werkplek waaraan beroepsmatige blootstelling kan plaatsvinden.

In het voorliggende rapport bespreekt de commissie de gevolgen van blootstelling aan diethyleenglycol en presenteert zij een gezondheidskundige advieswaarde voor deze stof. De conclusies van de commissie zijn gebaseerd op wetenschappelijke publicaties die vóór september 2006 zijn verschenen.

Fysische en chemische eigenschappen

Diethyleenglycol (CAS-nummer 111-46-6) is een synthetische wateraantrekkende, viskeuze vloeistof, zonder kleur en vrijwel zonder geur, met een smeltpunt van –10 °C, een kookpunt van 246 °C en een dampspanning van <0,01 kPa bij 25 °C. Diethyleenglycol wordt gebruikt als antivriesmiddel en als middel om water uit gassen te verwijderen. Ook zit het in smeermiddelen, remvloeistoffen, anti-schimmelmiddelen, inkt, cosmetica en verf. Diethyleenglycol wordt tevens gebruikt als weekmaker en als uitgangsstof bij de synthese van chemische stoffen.

Blootstelling

Er zijn geen gegevens beschikbaar over de blootstelling van werknemers aan diethyleenglycol. Contact met ogen en huid zou kunnen optreden bij het bereiden van bijvoorbeeld remvloeistoffen of antivriesmengsels. Inademing van dampen is verwaarloosbaar vanwege de lage dampspanning van diethyleenglycol bij kamertemperatuur. Alleen bij gebruik bij hogere temperaturen en bij zodanig gebruik dat aërosolen ontstaan, kan inademing van diethyleenglycol belangrijk worden.

Monitoring

Voor het meten van diethyleenglycol in lucht is een gedeeltelijk gevalideerde methode beschikbaar (NIOSH-methode 5523). Deze methode is gebaseerd op gaschromatografie met vlam-ionisatiedetectie. Daarnaast bestaan er methoden voor de meting van diethyleenglycol in bloedserum, bloedplasma en urine.

Grenswaarden

Er is geen wettelijke grenswaarde in Nederland voor de inhalatoire blootstelling aan diethyleenglycol. Er is ook geen norm vastgesteld door de Europese Commissie. In het Verenigd Koninkrijk, Duitsland, Denemarken en Zweden zijn grenswaarden vastgesteld van respectievelijk 101 mg/m^3, 44 mg/m^3, 11 mg/m^3 en 45 mg/m^3 (gemiddeld over een achturige werkdag). Daarnaast geldt in Zweden een grenswaarde van 90 mg/m^3, gemiddeld over een werkperiode van 15 minuten, en heeft diethyleenglycol een huidnotatie. In de Verenigde Staten heeft de American Industrial Hygiene Association een Workplace Environmental Exposure Limit van 10 mg/m^3 (gemiddeld over een achturige werkdag) voor de inademing van aërosoldeeltjes van diethyleenglycol.

Kinetiek en toxisch werkingsmechamisme

Aangenomen wordt dat diethyleenglycol snel wordt opgenomen na orale inname en na inademing van dampen. Voor mensen zijn over de opname via de huid geen gegevens bekend. Berekening met het model SkinPerm laat zien dat diethyleenglycol door de huid dringt met een maximale snelheid van 0,10 mg/cm^2/uur.

Na orale toediening aan proefdieren wordt diethyleenglycol snel door het lichaam opgenomen en verdeelt deze stof zich over alle organen en weefsels.

Diethyleenglycol wordt in het lichaam omgezet in 2-hydroxyethoxyazijnzuur. De stofwisseling van diethyleenglycol in oxaalzuur is een onbelangrijke metabole route, zo die al bestaat. Zowel diethyleenglycol als 2-hydroxyethoxyazijnzuur worden snel uitgescheiden met de urine. Na orale toediening van diethyleenglycol wordt 45 tot 70 procent onveranderd in de urine teruggevonden en 11 tot 37 procent als 2-hydroxyethoxyazijnzuur. Bij hoge concentraties in het bloed bereikt de omzetting van diethyleenglycol een plateau door verzadiging van de stofwisseling. Het product 2-hydroxyethoxyazijnzuur wordt verantwoordelijk gehouden voor de waargenomen schadelijke effecten op de nieren.

Effecten bij mensen

Er zijn geen gegevens over de effecten bij mensen van inhalatoire blootstelling aan diethyleenglycol. Er zijn ook geen gegevens over irriterende effecten op de ogen noch over sensibiliserende eigenschappen van deze stof. Op de huid geeft diethyleenglycol nauwelijks irritatie.

Inname van diethyleenglycol is gevaarlijk. Een eenmalige hoeveelheid van 0,5 tot 1 gram diethyleenglycol per kilogram lichaamsgewicht is vermoedelijk al fataal. De eerste verschijnselen zijn misselijkheid, braken, hoofdpijn, veel plassen, buikpijn en pijn in de rug. Binnen één tot twee dagen onstaat ernstige verzuring met toename van het aantal anionen in het bloed. Vervolgens gaat de nierfunctie achteruit en nemen in het bloed de activiteit van de aminotransferasen, de creatinineconcentratie en het aantal witte bloedcellen toe. Indien onbehandeld, kan de nierwerking stoppen en de dood volgen door acuut nierfalen. Bij autopsie zijn beschadigingen gevonden in de nieren en de lever.

Mensen blijken ongeveer tien keer zo gevoelig te zijn voor de schadelijke effecten van diethyleenglycol als knaagdieren. Zowel bij mens als dier zijn de nieren en de lever de doelorganen. Bij mensen zijn bovendien neurotoxische effecten beschreven waaronder aangezichtsverlamming en verlamming van de ledematen. Bij neuropathologisch onderzoek van overledenen heeft men ernstige beschadigingen gevonden aan myeline en axonen van aangetaste zenuwen.

Effecten bij proefdieren

Diethyleenglycol heeft geen of slechts geringe irriterende effecten op de huid en de ogen van proefdieren. In onderzoek bij cavia’s is geen sensibilisatie vastgesteld.

Orale toediening van diethyleenglycol veroorzaakt bij proefdieren een betrekkelijk milde vergiftiging. Muizen en ratten zijn minder gevoelig dan konij-

nen, katten en honden. Het meeste onderzoek is gedaan met knaagdieren. Kortdurende toediening van diethyleenglycol aan knaagdieren kan leiden tot beven, inactiviteit, slaap, recht opstaan van de haren en beschadiging van het netvlies. In het bloed neemt de activiteit van aminotransferasen toe en microscopisch kan men veranderingen in de hartspier zien. Bij hoge dosis gaat de nierfunctie achteruit en kan het dier overlijden.

In een onderzoek uit 1976 vond men oxalaatkristallen in de urine van ratten die dagelijks werden gevoerd met 234 tot 292 mg diethyleenglycol per kilogram lichaamsgewicht. Bovendien nam het urinevolume toe na een waterbelastingtest. Toediening van 105 mg diethyleenglycol per kilogram lichaamsgewicht per dag vergrootte bij mannetjesratten de hoeveelheid oxaalzuur in de urine met 13 tot 23 procent. Bij hoge doses vanaf 1550 mg diethyleenglycol per kilogram lichaamsgewicht per dag zag men hydropische degeneratie van de nieren en tubulaire necrose. Volgens de onderzoekers werd minder dan 0,5 procent van de toegediende hoeveelheid diethyleenglycol omgezet in oxaalzuur.[1] Gezien het feit dat diethyleenglycol niet of nauwelijks wordt gemetaboliseerd tot oxalaat, vermoedt de commissie dat de kristallen die bij de ratten zijn gevonden, geen oxalaatkristallen waren of althans nauwelijks oxalaat bevatten. De identiteit van de gevonden ‘oxalaatkristallen’ is derhalve onzeker. Bij fatale vergiftigingen van mensen zijn nooit oxalaatkristallen gevonden bij pathologisch onderzoek na overlijden. Wel is tweemaal een amorf, calciumhoudend neerslag beschreven.

De ‘International Agency for Research on Cancer’ heeft diethyleenglycol niet geëvalueerd. Verschillende onderzoekers hebben laten zien dat diethyleenglycol niet kankerverwekkend is wanneer het op de huid wordt gebracht.

Diethyleenglycol is niet genotoxisch *in vitro*. *In vivo* zijn bij proefdieren wel aanwijzingen gevonden voor genotoxiciteit, maar alleen bij hoge doses die ook toxisch zijn voor het moederdier. De commissie ziet dit niet als een teken van genotoxiciteit. Er zijn geen relevante effecten op de voortplanting en het nageslacht waargenomen bij proefdieren.

Evaluatie en advies

De effecten van diethyleenglycol op de nieren vormen het uitgangspunt voor de afleiding van de advieswaarde. Sleutelonderzoek is dat van Gaunt *et al.* (1976)[1] die ratten gedurende veertien weken blootstelden aan 0 tot 4 procent diethyleenglycol in hun voer. In een tweede experiment kregen ratten gedurende 225 dagen 0 tot 2 procent diethyleenglycol in hun voer. De onderzoekers vonden dat diethyleenglycol schade aanrichtte aan de nieren: het aantal dieren met oxalaatkristallen in de nieren nam toe, het volume van de urine na een waterbelastingstest nam

toe, en hydropische degeneratie van de nieren en tubulaire necrose traden op. Voor de toename van het aantal dieren met oxalaatkristallen en voor de toename van het volume van de urine komen de resultaten tussen de mannetjes en de vrouwtjes niet overeen en ziet de commissie geen dosis-responsrelatie. Bovendien twijfelt de commissie over de identiteit van de gevonden kristallen omdat diethyleenglycol niet of nauwelijks wordt gemetaboliseerd tot oxalaat. Daarom heeft de commissie ervoor gekozen de 'Health-Based Recommended Occupational Exposure Limit' (HBROEL) af te leiden uit de 'No Observed Adverse Effect Level' (NOAEL) gebaseerd op de histopathologische beschadigingen. In het veertien weken durende onderzoek begon hydropische degeneratie op te treden bij orale inname van 1550 mg diethyleenglycol per kilogram lichaamsgewicht per dag. Hieruit concludeert de commissie dat voor mannetjesratten de NOAEL voor chronische blootstelling 300 mg per kilogram lichaamsgewicht per dag is. Deze waarde heeft de commissie als uitgangspunt gekozen voor de afleiding van de HBROEL.

De commissie gaat er van uit dat de biologische beschikbaarheid na inademing en orale inname honderd procent is en dat het lichaamsgewicht van een werknemer zeventig kilogram is. Ook oordeelt de commissie dat mensen ongeveer tien keer zo gevoelig zijn voor de schadelijke effecten van diethyleenglycol als ratten. Daarom past de commissie een factor tien toe voor dit verschil in gevoeligheid. Daarnaast past de commissie een factor drie toe voor interindividuele verschillen in gevoeligheid. Dit geeft een totale onzekerheidsfactor van dertig. Uit de NOAEL van 300 mg per kilogram lichaamsgewicht per dag leidt de commissie een waarde af van 10 mg/kg lichaamsgewicht/dag, dit is 700 mg/werknemer/dag. Uitgaande van de inademing van 10 m^3 lucht gedurende een werkdag van acht uur komt dit overeen met een advieswaarde van 70 mg/m^3 (8-uurs tijdgewogen gemiddelde) voor diethyleenglycol in de lucht op de werkplek. De commissie merkt hierbij op dat deze advieswaarde betrekking heeft op de som van de concentratie diethyleenglycol in dampvorm en als aërosol.

Volgens de commissie kan blootstelling aan een aërosol effecten veroorzaken die vergelijkbaar zijn met de effecten van blootstelling aan inhaleerbaar en respirabel stof. Daarom is de commissie van mening dat gezondheidskundige advieswaarden voor de beroepsmatige blootstelling aan inhaleerbaar en respirabel stof ook moeten gelden voor een aërosol van diethyleenglycol.

Berekening met het model SkinPerm laat zien dat diethyleenglycol door de huid dringt met een maximale snelheid van 0,10 mg/cm^2/uur. Hieruit volgt dat blootstelling van de huid aan diethyleenglycol belangrijk kan bijdragen aan de interne belasting. Om deze reden en omdat diethyleenglycol een aanzienlijke

systemische toxiciteit heeft, beveelt de commissie een huidnotatie aan voor diethyleenglycol.

Gezondheidskundige advieswaarde

De commissie GBBS van de Gezondheidsraad stelt bij beroepsmatige blootstelling aan diethyleenglycol een gezondheidskundige advieswaarde voor van 70 mg/m^3, gemiddeld over een achturige werkdag (8-uurs tijdgewogen gemiddelde). Deze advieswaarde heeft betrekking op de som van de concentratie diethyleenglycol in dampvorm en als aërosol.

Daarnaast adviseert de commissie dat gezondheidskundige advieswaarden voor de beroepsmatige blootstelling aan inhaleerbaar en respirabel stof ook moeten gelden voor een aërosol van diethyleenglycol. De commissie beveelt tevens een huidnotatie aan voor diethyeenglycol.

Executive summary

Scope

At request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands recommends health-based occupational exposure limits for the concentration of toxic substances in the air in the workplace. These recommendations are made by the Council's Dutch Expert Committee on Occupational Standards (DECOS).

In this report, the committee discusses the consequences of occupational exposure to diethylene glycol and recommends a health-based occupational exposure limit. The committee's conclusions are made on scientific papers published before September 2006.

Physical and chemical properties

Diethylene glycol (CAS number 111-46-6) is a synthetic colourless, hygroscopic, viscous, practically odourless liquid with a melting point of -10 °C, a boiling point of 246 °C and a vapour pressure of <0.01 kPa at 25 °C.

Diethylene glycol is used for de-icing, industrial drying, textile softening, as an anti-freeze agent, as an intermediate in chemical syntheses, and as a component of brake fluids, lubricants, mould-release agents, and inks. It is also used as a plasticizer for cork, paper, adhesives, packaging materials, and coatings.

Exposure

Actual exposure data for workers are not available. Contact with the skin and eyes are likely routes of exposure during industrial handling, for instance during the manufacture of brake fluids or antifreeze mixtures. Exposure by inhalation of vapours is negligible due to diethylene glycol's low vapour pressure at room temperature. Inhalation exposure may become significant when aerosols are formed or when diethylene glycol is heated during industrial processing.

Monitoring

The National Institute for Occupational Safety and Health (NIOSH) recommends capillary gas chromatography with flame ionisation detection as the method for monitoring diethylene glycol in air after collection of vapour and aerosol particles with XAD-7 OVS sampler tubes (NIOSH method 5523).

Several methods exist for the measurement of diethylene glycol in blood plasm, blood serum, and urine. These are based on gas chromatography with flame ionisation detection or mass spectrometry as the main methods of detection and quantification.

Limit values

At present, there is no occupational exposure limit for diethylene glycol in the Netherlands. Also, there is currently no limit value established by European Commission. The United Kingdom, Germany, Denmark and Sweden have set occupational exposure limits for diethylene glycol of 101 mg/m^3, 44 mg/m^3, 11 mg/m^3 and 45 mg/m^3, respectively, as an 8-hour time-weighted average. Furthermore, Sweden has established a 15-minute short-term exposure limit of 90 mg/m^3. A skin notation is attached to diethylene glycol in Sweden. The American Industrial Hygiene Association has a Workplace Environmental Exposure Limit of 10 mg/m^3 (8-hour time-weighted average) for diethylene glycol as an aerosol.

Kinetics and mechanism of action

In man, diethylene glycol is assumed to be readily absorbed following inhalatory exposure and after oral intake. No data have been found on the dermal absorption in man. According to the model SkinPerm, the maximal skin permeation is 0.10 mg/cm^2/hour under steady-state conditions.

In animals, absorption of diethylene glycol after oral administration is rapid and distribution occurs to all organs and tissues. Diethylene glycol and its metabolites are readily excreted in the urine. Depending on the dose administered, ca 45 to 70% of the dose is excreted unchanged in the urine within 48 hours after dosing, and 11 to 37% as 2-hydroxyethoxyacetic acid after oxidative metabolism. Saturation of metabolism was observed at high doses. Metabolic breakdown of diethylene glycol into oxalate appears to be a minor route in rats, if at all existent. It is believed that a metabolite of diethylene glycol rather than the parent compound itself is responsible for the adverse effects on the kidney.

Effects - Human toxicity data

There is no information available on the effects of inhalatory exposure to diethylene glycol in humans. No data were found on eye irritating or sensitising properties of diethylene glycol in humans. Diethylene glycol has mild if any irritating effects on the human skin.

The main reported health hazard of diethylene glycol is demonstrated by acute oral exposure. The administration of drugs formulated in diethylene glycol has learned that ingestion of 0.5 to 1 g/kg body weight can lead to severe intoxication with acute renal failure and potentially fatal. Initial effects include nausea, vomiting, headache, polyuria, and abdominal and back pains. Laboratory analysis reveals metabolic acidosis with increased anion gap, increased level of the serum aminotranferases, increased serum creatinine, and elevated white blood cell count. If untreated, renal failure may develop within a few days, followed by coma, convulsions and death in severe cases. Autopsy has revealed lesions in the kidneys and the liver.

Man appears to be about ten times more sensitive towards the toxic effects of diethylene glycol than the animal species used in toxicity studies. Both animal experiments and accidents in man have shown that the kidneys and the liver are the target organs. In man, neurotoxic effects have been reported and include facial paresis and ascending paralysis. Severe demyelination and axonal damage of central and peripheral nerves have been found post mortem.

Effects - Animal toxicity data

Diethylene glycol has no or only slight skin and eye irritating effects. In a maximisation study with guinea pigs, diethylene glycol caused no sensitisation.

Diethylene glycol has relatively low acute toxicity in animals, mice and rats being the most resistant, and rabbits, cats and dogs being more sensitive. Follow-

ing acute oral administration of diethylene glycol, the clinical signs of toxicity resemble those reported in humans. Oral short-term exposure of rodents results in tremor, lethargy, piloerection, decreased renal function, retinopathy (histopathological as well as electrophysiological), increased serum aspartate aminotransferase activity, microscopical and ultrastructural myocard changes. At high doses, renal failure may develop and lead to death.

In a rat study from 1976, oxalate crystals were found in the urine at the dietary dose level of 0.4% diethylene glycol (230-290 mg/kg bw/day). In the males, the urine volume after a concentration test was increased.[1] At 0.17% diethylene glycol (105 mg/kg bw/day), a 13-23% increase of urinary oxalate excretion was found in the males. At dose levels of 2.0% (1,550 mg/kg bw/day) and higher, hydropic degeneration of the kidneys and tubular necrosis were observed as the histopathological findings. According to the authors, less than 0.5% of the daily intake of diethylene glycol was metabolised into oxalate. In view of the fact that diethylene glycol is not or barely metabolised into oxalate, the committee expects that the crystals found in this study were mistaken as oxalate crystals, and did not or barely contain oxalate. The identity of the oxalate crystals found, therefore, is uncertain. In man, oxalate crystals have not been found in post-mortem examinations carried out in fatal cases of acute diethylene glycol ingestion, but amorphous calcium deposits have been described twice.

The International Agency for Research on Cancer has not evaluated diethylene glycol. Several studies showed that diethylene glycol is not carcinogenic after dermal administration.

The available data indicate that diethylene glycol was negative in *in vitro* tests for genotoxicity. Positive results were obtained in *in vivo* genotoxicity studies, however, only at high toxic doses of diethylene glycol. The committee does not consider this as an indication of genotoxicity.

Several reproduction toxicity studies and developmental toxicity studies have been carried out. From these studies, the committee concludes that, if diethylene glycol has any reproduction toxic properties, these will probably only be expressed at high dose levels causing biologically relevant maternal toxicity.

Evaluation and advice

The effects of diethylene glycol on the kidneys after prolonged oral exposure are considered as the critical effects. Key study is the oral exposure study in rats carried out by Gaunt *et al.* (1976).[1] These investigators found oxalate crystaluria, increased urine volumes after concentration tests, and hydropic degeneration and tubular necrosis as the histopathological findings. For the crystaluria and

increased urine volumes after concentration tests, the results in the male and the female rats are inconsistent, and no clear dose-response relationships can be observed for these effects. In addition, the committee has doubts about the identity of the crystals found. Therefore, the committee concludes that the Health-Based Recommended Occupational Exposure Limit (HBROEL) must be derived from the No Observed Adverse Effect Level (NOAEL) based on the histopathological findings. Hydropic degeneration of the kidneys started to occur at oral dose levels of 1,550 mg/kg bw/day for 14 weeks and was not seen at 300 mg/kg bw/day. The committee concludes that the NOAEL for hydropic degeneration is 300 mg/kg bw/day (0.4% diethylene glycol in food) in the male rats.

Starting-point for the derivation of the HBROEL is the oral NOAEL of 300 mg/kg bw/day from the 14-week experiment of Gaunt *et al.* (1976).[1] An assessment factor of ten is used for interspecies differences as man appears to be about ten times more sensitive for the adverse effects of diethylene glycol than laboratory animals. For intraspecies differences, the default factor of three is used. With the overall assessment factor of 30 and assuming 70 kg as the average body weight of a worker and 10 m^3 as the average respiratory volume per 8-hour working day, the Dutch Expert Committee on Occupational Standards recommends an inhalatory HBROEL of 70 mg/m^3 as an 8-hour time-weighted average concentration (8-hour TWA). This value applies to the sum of the concentrations of diethylene glycol existing as a vapour and as an aerosol.

According to the committee, exposure to an aerosol can have effects that are comparable to the effects of exposure to inhalable and respirable dust. Therefore, it is the committee's opinion that health-based occupational exposure limits for inhalable and respirable dust must be applied to aerosols of diethylene glycol.

Although skin absorption has been demonstrated in rats, these data are insufficient to assess whether a skin notation is needed. Skin permeation data obtained with the model SkinPerm indicate that dermal exposure may substantially contribute to the body burden of diethylene glycol. Because of significant systemic toxicity, the committee recommends a skin notation for diethylene glycol.

Health-based recommended occupational exposure limit

The Dutch Expert Committee on Occupational Standards recommends a health-based occupational exposure limit of 70 mg/m^3 as an eight-hour time-weighted average concentration (8-hour TWA), applying to the sum of the concentrations of diethylene glycol existing as a vapour and as an aerosol.

The committee also recommends to apply health-based occupational exposure limits for inhalable and respirable dust to aerosols of diethylene glycol. In addition, the committee recommends a skin notation for diethylene glycol.

Chapter 1

Scope

1.1 Background

At the request of the Minister of Social Affairs and Employment (annex A), the Dutch Expert Committee on Occupational Standards (DECOS), a committee of the Health Council of the Netherlands, performs scientific evaluations of the toxicity of substances that are used in the workplace. The purpose of these evaluations is to recommend health-based occupational exposure limits for concentrations in the air, provided the database allows the derivation of such values. In the Netherlands, these recommendations serve as the basis for setting public occupational exposure limits by the minister.

Committee and procedure

The present document contains the assessment of DECOS, hereafter called the committee, of the health hazard of diethylene glycol. The members of the committee are listed in annex B. The first draft of this document has been prepared by G. Schaafsma and C. de Heer of the Toxicology Division of TNO Quality of Life, Zeist, the Netherlands.

In 2006, the President of the Health Council released a draft of the report for public review. The individuals and organizations that commented on the draft are listed in annex C. The committee has taken these comments into account in deciding on the final version of the report.

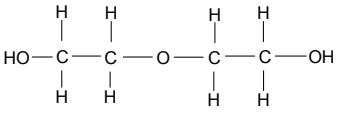
Data

The committee's recommendations on the health-based occupational exposure limit of diethylene glycol have been based on scientific data, which are publicly available. Data were obtained from the online databases Toxline, Medline/Pubmed, Current Contents and Chemical Abstracts, using the key words diethylene glycol and/or CAS no 111-46-6 in combination with: use, expos*, kinetic*, toxic*, animal, human, adverse effects. The literature from this search was selected based on titles and abstracts. The last search was performed in September 2006. Furthermore, relevant literature on diethylene glycol cited in the IUCLID dataset[2] was consulted.

Chapter **2**

Identity, properties and monitoring

2.1 Chemical identity

Chemical name	Diethylene glycol
Synonyms	2-(2-hydroxyethoxy)ethane-2-ol; bis(2-hydroxyethyl) ether; Brecolane ndg; Deactivator H; DEG; Dicol; Diglycol; β,β'-Dihydroxyethyl ether; Dissolvant APV; Ethanol, 2,2'-oxydi-; Ethylene diglycol; Glycol ether; Glycol ethyl ether; 3-Oxa-1,5-pentanediol; 2,2'-Oxybisethanol; 2,2'-Oxydiethanol; TL4N; 2,2'-dihydroxyethyl ether; Dihydroxydiethyl ether; 3-Oxapentane-1,5-diol; 2,2'-Oxyethanol; 2,2'-Oxydiethanol; Deactivator E
Molecular formula	$C_4H_{10}O_3$
Structural formula	
CAS number	111-46-6
EINECS number	203-872-2
EEC number	603-140-00-6
RTECS number	ID5950000

2.2 Physical and chemical properties

Physical description	colourless, hygroscopic, viscous, practically odourless liquid; sharply sweetish with bitter aftertaste
Molar mass	106.12 g/mol
Melting point	-10 °C
Boiling point	246 °C
(relative) density	1.115 g/cm^3 = 1115 kg/m^3 (20 °C/4 °C)
Solubility (in water, ethanol, ether)	miscible with water, ethanol, ether, acetone and ethylene glycol
Log $P_{octanol/water}$	-1.98
Vapour pressure	< 0.01 kPa at 20 °C; saturated vapour contains < 100 ppm, that is < 440 mg/m^3
Relative vapour density (air = 1)	3.66
Flash point	124 °C (closed cup); 143 °C (open cup)
Odour threshold	essentially odourless; reported threshold in aqueous solutions: 3.37 g/l
Conversion factor (20 °C, 101.3 kPa)	1 mg/m^3 = 0.227 ppm 1 ppm = 4.414 mg/m^3

2.3 EU Classification and labeling

Based on Annex I of Council Directive 67/548/EEC (as adapted to technical progress for the 29th time by Directive 2004/73/EC), diethylene glycol is classified with Xn; R22 (harmful if swallowed). No specific concentration limits are derived for the classification of diethylene glycol.[3]

2.4 Analytical methods

2.4.1 Environmental monitoring

In ambient air at room temperature and atmospheric pressure, diethylene glycol may exist as a vapour and as aerosol particles. For monitoring vapour and aerosol particles, the partially evaluated NIOSH method 5523 is available with a XAD-7 OVS tube as the sampling tube. For monitoring aerosol particles, the particle size should be determined first. Depending on the particle size, the inhalable aerosol fraction can be measured by the gravimetric sampling technique. In the Netherlands, personal inhalable particulates are usually sampled with the Dutch ‘PAS6’ sampling head mounted near the breathing zone of the worker.

Table 2.1 gives a summary of the methods reported in the literature for the analysis of diethylene glycol. Gas chromatography with flame ionisation detection is the technique used to determine diethylene glycol in air.[4] Gas chromatography with fourier transform infrared spectrometry has been used to detect diethylene glycol in aqueous matrices.[5]

Table 2.1 Analytical methods for determining diethylene glycol in environmental samples.

Sample matrix	Sampler	Sample preparation	Assay procedure	LOD, LOQ[b]	Reference
Air (NIOSH method 5523)[a]	XAD-7 OVS tube (glass fiber filter, 13-mm; XAD-7, 200mg/100mg)	Collection on glass fibre filter / XAD-7 sorbent; extraction with methanol	GC-FID[b]	LOD: 16 µg/sample LOQ: 48 µg/sample	NIOSH 1996[4]
Air (OSHA method Diethylene Glycol)	OSHA Versatile Sampler (OVS-7), 13-mm XAD-7 tube (270/140 mg), glass fiber filter enclosed	Collection on glass fibre filter / XAD-7 sorbent; extraction with methanol	GC-FID[b]	not reported	OSHA 2002[6]
Aqueous matrices (EPA method 8430)	-	Direct aqueous injection	GC-FT-IR[b]	Not reported	EPA 1996[5]

[a] Requires samples to be packed in dry ice for shipment.
[b] GC: gas chromatography; FID: flame ionisation detection; FT-IR: fourier transform infrared spectrometry; LOD: limit of detection; LOQ: limit of quantitation.

2.4.2 *Biological monitoring*

Table 2.2 contains methods for the the measurement of diethylene glycol in biological samples. No information is available on the assay of diethylene glycol in faeces, adipose tissue, or human milk.

Table 2.2 Analytical methods for determining diethylene glycol in biological samples.

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Serum	Deproteinisation by ultrafiltration	GC	25 mg/l	Williams *et al.* 2000[7]
Serum	Extraction after basidification; derivatisation with heptafluorobutyric pyridine-ethanol	GC with FPEC detection or MS	Not reported	Brooks 1984[8]
Serum	Addition of the internal standard	GC-FID on a highly polar stationary phase (Nukol)	Not reported	Edinboro *et al.* 1993[9]
Plasma	Addition of 1,3-propylene glycol as the internal standard; extraction; derivatisation with pivalic acid	GC-MS	< 0.01 g/l	Maurer *et al.* 1986[10]; Maurer and Kessler 1988[11]
Whole blood, serum	Deproteinisation, derivatisation	GC-MS	18 mg/l	Gembus *et al.* 2002[12]
Plasma, urine	Addition of 1,3-propanediol as the internal standard; deproteinisation; evaporation; derivatisation with pivalic acid anhydride	GC[b]	0.01 g/l LOQ[a]: 0.1 g/l	Maurer *et al.* 2001[13]
Urine	Addition of the internal standard	NMR spectroscopy	Not reported	Lenk *et al.* 1988[14]

[a] GC: gas chromatography; MS: mass spectrometry; FID: flame ionisation detection; FPEC: frequency-pulsed electron-capture; NMR: nuclear magnetic resonance; LOQ: limit of quantitation.

[b] The quantification has been validated according to the criteria established by the *Journal of Chromatography B*.[15]

Chapter 3

Sources

3.1 Natural occurrence

Diethylene glycol is not known to occur as a natural product.

3.2 Man-made sources

3.2.1 Production

The IUCLID dataset[2] mentions a diethylene glycol quantity of 100-500 thousand tonnes for Europe. It is not clear whether this concerns import, production or both. In the IUCLID dataset it is not specified whether diethylene glycol is produced in open or closed systems. Diethylene glycol is produced via a non-catalytic reaction between ethylene oxide and water at high pressure temperature. The resulting crude ethylene glycols are dried. The water-free glycol mixture is subsequently fractionated by vacuum distillation into mono, di and triethylene glycol.

Biodegradation of polyethylene glycols results in chain shortening with concomitant formation of ethylene glycol and diethylene glycol in nature.[16]

3.2.2 Use

Because diethylene glycol has relatively low chemical reactivity and does not ignite at ambient temperatures, it has been used for a long time for various applications.[17-24]

Diethylene glycol is used:

- as an intermediate in the production of the explosive diethylene glycol nitrate (increases freeze-thaw stability)
- in the manufacture of diethylene glycol esters and ethers
- in the manufacture of certain resins (produces flexibility of polyesters, for example, as a softening agent for vinyl resins in rayon finishing and as an alcohol component for polyester resins in the plastics and lacquer industries)
- as a textile finishing agent for wool, rayon, cotton, and silk
- as a de-icer for aircrafts and take-off/landing runways
- as an anti-freeze component in sprinkler systems and in water seals in gas tanks (a 40% solution in water freezes at -18 °C and a 50% solution at -28 °C)
- for industrial drying and desulfurising of gases (*e.g.*, natural gas)
- as a constituent of brake fluids (rubberswell inhibitor, increases water tolerance of the fluid)
- as a lubricant (*e.g.*, for synthetic polymers)
- in mildew removers
- as a moistening/hygroscopic agent in the production of composition corks, pates, paper, glues, gelatine, cheese, gum drops and in some cigarette tobaccos
- as a solvent for water insoluble substances, oil based paint, dyes, inks, lacquers and cosmetics
- as a solvent for food flavouring, such as ice cream
- as a plasticizer for cork, adhesives, paper, packaging materials, adhesive bandages and coatings
- as a constituent in textile soaps and cosmetic creams.

Chapter 4

Exposure

4.1 General population

The principal route of exposure to diethylene glycol for the general population is ingestion of food containing small amounts of diethylene glycol. In sweets and in meat pies, diethylene glycol migrating from cellulose films has been detected. Small amounts of diethylene glycol are found in gelatine and tobacco (actual concentrations: 0.3-2.2%), cheese (actual concentration: 0.6%) and gum drops (1-30 mg/kg).[17,22] After preservation with ethylene oxide small amounts of diethylene glycol can be detected in wheat.[25] Furthermore, common exposures of adults to diethylene glycol result from dermal contact during use of antifreeze mixtures at home.[26] Exposure may also occur from pharmaceuticals, cosmetics, and toothpastes[27] that contain diethylene glycol.

4.2 Working population

Actual exposure data for workers are not available. Contact with the skin and eyes are likely routes of exposure during industrial handling, for instance during the manufacture and handling of anti-freeze mixtures.[26] Inhalation exposure may become significant when aerosols are formed or during heating of diethylene glycol. Diethylene glycol exposure from inhalation of vapours at room temperatures is negligible due to its low vapour pressure.[17]

Chapter 5

Kinetics

5.1 Absorption

Inhalation

No studies on the absorption of diethylene glycol after inhalation exposure are available. Because of the polar and hygroscopic characteristics of diethylene glycol, it can be assumed that diethylene glycol in vapour form is absorbed soon after it enters the upper respiratory passages. Diethylene glycol in aerosol form is probably also absorbed in the upper respiratory passages.[18]

Dermal

Mathews *et al.* (1991)[26] dermally exposed adult male Fischer 344 rats to ^{14}C-diethylene glycol (50 mg/12 cm^2). Diethylene glycol was slowly absorbed (open, but protected application). Approximately 3% of the applied dose of 50 mg/12 cm^2, that is 1.5 mg, was recovered from the urine each day for a period of 72 hours. After three days, a cumulative total of 9.1% of the dose of 50 mg/12 cm^2 was recovered in excreta (urine, faeces and as CO_2), and 0.9% in tissues. No further details were given in this study.

Calculation with the model SkinPerm[28] indicates that the maximal skin permeation is 0.10 mg/cm^2/hour under steady-state conditions when skin absorption equals systemic delivery. This model also predicts a significant latency period of

approximately three hours after onset of skin exposure before systemic delivery starts to occur.

Oral

In rats, single oral gavage doses of 1; 5; and 10 ml/kg bw (respectively 1.12; 5.6; and 11.2 g/kg bw) ^{14}C-diethylene glycol were rapidly and almost completely absorbed from the gastrointestinal tract.[22] Maximal plasma concentrations (radioactivity) were found after 30-120 min (96% of the dose was absorbed), 30-100 min (91% of the dose was absorbed) and 150-240 min (96% of the dose was absorbed) for the doses of 1.12; 5.6; and 11.2 g/kg bw ^{14}C-diethylene glycol, respectively. In animals receiving 1.12 g/kg bw the oral absorption rate was linear for 10-14 min, but after a dosage of 5.6 and 11.2 g/kg bw only for 4 min and 2 min after dosing, respectively. The initial linear increase of ^{14}C radioactivity in blood was followed by a slower, irregular pattern of increase, indicating that the rate of entry into the bloodstream is much faster than that of its distribution into the body fluids and tissues.

5.2 Distribution

After single oral gavage doses of 1, 5 and 10 ml/kg bw respectively (1.12; 5.6; and 11.2 g/kg bw) ^{14}C-diethylene glycol to rats[22], radioactivity was rapidly distributed from the blood into the organs and tissues in the order kidneys > brain > spleen > liver > muscle > fat and rapidly excreted in the urine (maximal plasma concentrations of ^{14}C determined after 25-120 min correspond to 5-10% of the dose administered). The apparent volume of distribution was determined at 1 l/kg, indicating distribution into total body water and tissues. The blood-brain barrier was crossed by diethylene glycol, but the uptake in the brain was slower: after 3-4 hour maximal ^{14}C concentrations were found. The high diethylene glycol concentrations in the rat brain were responsible for the narcotic effects of diethylene glycol. These were seen in all animals after 20 min and terminated after 6-8 hours. The lowest ^{14}C radioactivity was found in fat tissue in agreement with diethylene glycol's low lipophilicity. It was also shown that the concentration of ^{14}C in the kidneys was temporarily (20 minutes to 4 hours) higher than in blood, which reflects their function as the main excretory organ for diethylene glycol.

Retention of the radioactivity in rat tissues was minimal 72 hours post dosing, with the highest tissue to blood ratios (4-5) in the liver after a single oral gavage dose of 50 mg/kg bw. There was little or no difference between the distri-

bution of intravenous and oral doses of diethylene glycol at a level of 50 mg/kg bw.[26]

In order to test whether ^{14}C activity accumulated in the rat, oral gavage doses of 1, 5 and 10 ml/kg bw (respectively 1.12, 5.6 and 11.2 g/kg bw) ^{14}C-diethylene glycol were given and the residual ^{14}C activity in organs and tissues determined after 5 days. A total of 0.25-1.2% of the applied ^{14}C activity was found in the kidneys, liver, stomach, intestine, heart, lung, testes and urinary bladder, and 1.2-3.1% in muscle and fat tissue, skin and fur.[22]

5.3 Biotransformation

In animals, the postulated pathway of diethylene glycol metabolism (purity of diethylene glycol batch tested was >99.9%) involves oxidation by alcohol dehydrogenase to 2-hydroxyethoxy acetaldehyde which is further oxidized by aldehyde dehydrogenase to 2-hydroxyethoxyacetic acid (2-HEAA).[29] Both enzymatic reactions require NAD as the cofactor. Further oxidation of 2-HEAA to 2,2'-oxydiacetic acid (diglycolic acid) may occur. On acidification, 2-oxo-1,4-dioxan is formed in urine from 2-HEAA.[30] In Figure 1, the possible pathways of diethylene glycol metabolism in rats are shown.

In rats exposed to a single oral dose of 1 ml/kg bw (1.12 g/kg bw) diethylene glycol (purity not specified), 45-75% of the total dose was excreted into the urine within one day and 60-70% of urinary ^{14}C radioactivity consisted of unmetabolised diethylene glycol.[31] Lenk *et al.* (1988)[14] found that rats treated with oral doses of 1 ml/kg bw (1.12 g/kg bw) diethylene glycol (not specified whether ^{14}C-diethylene glycol was used; purity not specified) excreted 48% unmetabolised in the 48-hour urine with a half-life of 9 hours, and further 14% as 2-HEAA, accounting for 62% of the dose. The urinary excretion of diethylene glycol and 2-HEAA appeared to be complete after 48 hours. After oral doses of 5 ml/kg bw (5.6 g/kg bw) diethylene glycol, 38% of the dose was excreted unmetabolised in the 48-hour urine and 11% as 2-HEAA, accounting for 49% of the administered dose. At this dose level, the urinary excretion of radioactivity was not complete after 48 hours. The fate of the remainder of the applied dose was not investigated by the authors.

In another study by Lenk *et al.* (1989)[30], rats given single oral doses of 1; 5; and 10 ml/kg bw (respectively 1.12; 5.6; and 11.2 g/kg bw) ^{14}C-diethylene glycol (radiochemical purity 97.9%) by gavage excreted an average of 63%, 61% and 68% of the doses as unchanged diethylene glycol, respectively, and an average of 30%, 21% and 16%, respectively, as 2-HEAA within 72 hours in the urine.[30] The

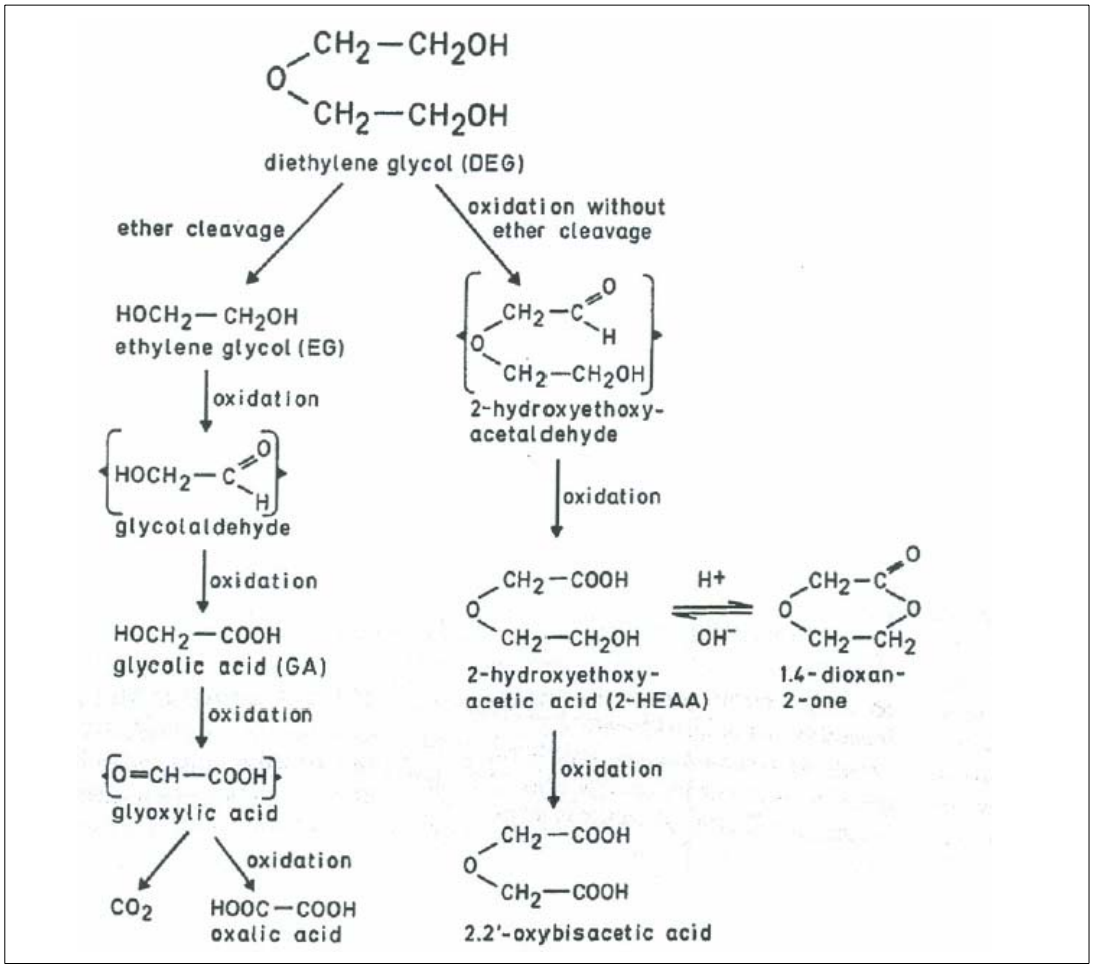


Figure 1. Hypothetical pathways of diethylene glycol metabolism in rats.[30] Under the proposition that the ether linkage of diethylene glycol is cleaved metabolically, two molecules are formed which are oxidized to give glycolic acid which can be further metabolised into CO_2 and oxalic acid. In man, cleavage of the ether linkage does not or insignificantly occur, and oxalic acid has not been found in diethylene glycol poisonings. Oxalic acid may also be formed after administration of diethylene glycol contaminated with ethylene glycol. Alternatively, if the ether linkage is not cleaved metabolically, diethylene glycol is oxidized into 2-HEAA and hypothetically into 2,2'-oxydiacetic acid (diglycolic acid). On acidification of urine containing 2-HEAA, 2-oxo-1,4-dioxan is formed.

total recoveries were 93%, 82%, and 84%, respectively, of the doses given. When the dose increased, the portion of diethylene glycol which was oxidized to CO_2 (elimination of total ^{14}C in expired air) decreased from 1.3% to 0.3%, indicating saturation kinetics for the oxidation of diethylene glycol to CO_2.

After single oral gavage and intravenous doses of diethylene glycol (purity 98%) given at levels of 50 mg/kg bw to rats, characterization of the urinary ^{14}C radioactivity (0 to 6-hour collections) indicated that both ways of administration yielded unchanged parent compound in the urine comprising 61-65% of recovered radioactivity and urinary 2-HEAA comprising 33-37% of recovered radioactivity[26]. Saturation of metabolism occurred after a 5,000 mg/kg bw dose. The profile of metabolites found in urine after intravenous and oral dosing was similar to that found for diethylene glycol supplied in drinking water given to rats at levels of 0.3; 1; and 3% (equivalent to 523; 1,583; and 8,191 mg/kg bw) for a 24 hour period. In addition, an oral gavage dose of diethylene glycol (500 mg/kg bw) administered to female Beagle dogs was excreted as unchanged parent compound and as 2-HEAA (with about 30% of the dose being converted to 2-HEAA), and 92% of the ^{14}C radioactivity was recovered from the urine within 72 hours.[26]

In rats, Winek *et al.* (1978)[21] found biological half-lives of 8 hours and 12 hours after oral doses of 6 and 12 ml/kg bw (6.7 and 13.4 g/kg bw) diethylene glycol, respectively, indicating that the plasma half-life was dose-dependent and that the metabolism and/or urinary elimination of diethylene glycol may become saturated.

From these studies, 2-HEAA appeared to be the major metabolite in rats and dogs. Consequently, the origin of the oxalate found in several animal studies with diethylene glycol has been questioned. Wolff *et al.* (1986)[31] suggested that the small amounts of oxalate found resulted from the oxidation of small amounts of ethylene glycol present as a contaminant in diethylene glycol. This is possible as diethylene glycol is fractionated from crude ethylene glycol mixtures during production. In early studies, the purity of diethylene glycol was not stated or measured.

Metabolic acidosis is not immediately seen in acute human diethylene glycol poisoning but may develop in about one day after ingestion.[32] The slow development of metabolic acidosis has been explained by the slow formation of ethylene glycol from diethylene glycol.[33] Alternatively, it may be explained by the slow formation of 2-HEAA from diethylene glycol. As the metabolism of diethylene glycol is not fully elucidated, the committee can not exclude the formation of small amounts of oxalate from diethylene glycol. It is the committee's opinion, however, and the generally accepted view[32,34], that the ether bond in diethylene glycol is not (or hardly) cleaved and that oxalate is a minor metabolite if at all existing in man. Therefore, the committee's judgement is that the oxalate crystals reported in several studies may be based on mistaken identity. For this reason,

the word ‘oxalate’ in oxalate crystals has been put between quotation marks whenever needed to indicate doubt about their identity.

5.4 Elimination

No data on elimination of diethylene glycol in humans were found in the literature. No information was available with regard to elimination of diethylene glycol in mother’s milk.

Animal

Single oral gavage doses of ^{14}C-diethylene glycol given at levels of 50 and 5,000 mg/kg bw to rats were excreted primarily in the urine within 72 hours (85% and 97%, respectively), with 55 to 60% appearing in the first 6 hour post dosing[26] Excretion in the faeces accounted for less than 1% of either dose. Conversion to carbon dioxide and exhalation occurred for 6% of the 50 mg/kg bw dose and for 1.5% of the 5,000 mg/kg bw dose. The profiles of excretion of radioactivity into urine and faeces were similar for ^{14}C-diethylene glycol supplied in drinking water to rats at levels of 0.3, 1 and 3% (equivalent to 523; 1,583; and at 8,191 mg/kg bw) for a 24-hour period: 84.6% (at 523 mg/kg bw) – 91.2% (at 8,191 mg/kg bw) was excreted via the urine, 7.2% (at 523 mg/kg bw) – 2.4% (at 8,191 mg/kg bw) was excreted as CO_2 (indicating some saturation of metabolism at the higher dose), and 1.2% (at 523 mg/kg bw) – 1.6% (at 8,191 mg/kg bw) was excreted in the faeces after 72 hours.[26]

In the study by Lenk *et al.* (1989)[30] described above, rats were given single oral gavage doses of 1; 5; and 10 ml/kg bw (1.12; 5.6; and 11.2 g/kg bw) ^{14}C-diethylene glycol. From the urinary excretion data elimination, half-lives of 6; 6; and 10 hours for the oral doses of 1.12; 5.6; and 11.2 g/kg bw ^{14}C-diethylene glycol were found. Only trace amounts of diethylene glycol (<1.3% of dose) and 2-HEAA (<0.7% of dose) were found in the 48-72-hour urine, indicating that diethylene glycol was excreted rapidly, that is within 48 hours. During the first 6; 9; and 18 hours after 1.12; 5.6; and 11.2 g/kg bw doses, respectively, the urinary elimination rate of ^{14}C was constant, indicating zero-order elimination. Urinary elimination kinetics became first-order after 6; 9; and 18 hours, respectively, with a half-life of 3 hours. The authors also found a linear relationship between the orally administered dose of diethylene glycol and the 0-24 hours urine volume for doses up to 15 ml/kg bw (16.7 g/kg bw), suggestive of osmotic diuresis. With increasing dose, the fraction oxidized to CO_2 (elimination of total ^{14}C in expired air) decreased from 1.3% to 0.3%, indicating saturation kinetics for the

oxidation of diethylene glycol to CO_2. Similar low amounts of ^{14}C label (2.2% to 0.7%) were present in faeces collected for 5 days, showing that faecal excretion of ^{14}C in the rat is a minor pathway. When various organs of the carcass were examined for residual ^{14}C activity 5 days after oral doses of ^{14}C-diethylene glycol, higher ^{14}C activity was found in liver, muscles, fat and skin compared with blood, intestine and kidney.

Heilmair *et al*. (1993)[22] exposed rats to single oral gavage doses of 1; 5; and 10 ml/kg bw (respectively 1.12; 5.6; and 11.2 g/kg bw) ^{14}C-diethylene glycol (radiochemical purity 97.5%). In this study 64; 87; and 91% of the ^{14}C activity in blood disappeared in 12-16 hours with a plasma half-life of approximately 3.4 hours, and in the following 20-36 hours the remaining 9; 5; and 4% with half-lives of 39; 45; and 49 hours for the doses of 1.12; 5.6; and 11.2 g/kg, respectively. A total 73-96% of ^{14}C-diethylene glycol activity in blood was excreted with the urine and 0.7-2.2% with the faeces. Urinary half-lives of 6 hours were determined for 1.12 and 5.6 g/kg bw doses, and of 10 hours for the 11.2 g/kg bw dose. Whereas doses of 1.12 gl/kg bw ^{14}C-diethylene glycol did not significantly alter the urine flow within 42 hours, doses of 5.6 and 11.2 g/kg bw ^{14}C-diethylene glycol caused a 7 to 11-fold increase in the urine volumes of the first 2 hours. After doses of 5.6 and 11.2 g/kg bw, elimination rate versus time was constant for 5 and 9 hours, respectively, indicating that diethylene glycol accelerated its renal elimination by inducing osmotic diuresis. Subsequently, the urinary excretion followed first-order kinetics with elimination half-lives of 3.6 hours. The renal elimination of the higher doses was accelerated so much, that the same ^{14}C activity (71.8%) which was excreted in 24 hours after doses of 1.12 g/kg bw, was excreted in 8 hours at the higher doses of 5.6 and 11.2 g/kg bw. After oral doses of 5.6 g/kg bw ^{14}C-diethylene glycol given to the rats (mean body weight: 336 g), the apparent distribution volume was 297 ml (0.9 l/kg), the total clearance of ^{14}C activity was 63 ml/hr, and the renal clearance of unmetabolised diethylene glycol was 66 ml/h. The ratio of $Cl_{diethylene\ glycol}$ to Cl_{inulin} was 0.64 indicating that diethylene glycol and its metabolite 2-HEAA were reabsorbed from the tubuli into the blood capillaries.

Diethylene glycol doses of 1 to 15 ml/kg bw (1.12 to 16.7 g/kg bw) caused a linear increase in the 24-hour urine volume, suggestive of an osmotic-diuretic effect. Oral doses of 17.5 ml/kg bw (19.5 g/kg bw) caused a further increase of the urine volume in only three out of ten animals, indicating a limitation of the renal excretory capacity. Diethylene glycol doses of 16.7 g/kg bw and 19.5 g/kg bw produced a fourfold increase in the volume of the 24-hour urine as compared to that of control animals. Not all the rats were able to compensate the loss of

25% of body water by drinking because of the narcotic effect caused by diethylene glycol.[22]

5.5 Possibilities for biological monitoring

Measurements of diethylene glycol in blood and urine provide good estimates of exposure because the larger part of the absorbed dose is excreted unchanged in urine (see also section 2.4.2).

5.6 Summary

Because of its rapid absorption after oral administration, it can be assumed that diethylene glycol is absorbed soon after it enters the upper respiratory passages. Dermally administered diethylene glycol (50 mg/12 cm^2) was slowly absorbed in rats (open, but protected application). Approximately 3% of 50 mg/12 cm^2 was recovered from the urine each day for a period of 72 hours. After three days a cumulative amount of 9.1% of the dose of 50 mg/12 cm^2 was recovered from excreta, and 0.9% from tissues. Calculation with the model SkinPerm indicates that the maximal skin permeation is 0.10 mg/cm^2/hour under steady-state conditions when skin absorption equals systemic delivery. This model also predicts a significant latency period of approximately three hours after onset of skin exposure before systemic delivery starts to occur.

Diethylene glycol is rapidly distributed from the blood into the organs and tissues in the order kidneys > brain > spleen > liver > muscle > fat. The lowest concentrations of ^{14}C activity were found in fat tissue. The concentration of ^{14}C in the kidneys was temporarily (20 min – 4 hours) higher than in blood, which reflects the function of the kidneys as the main excretory organ for diethylene glycol.

The suggested pathway of diethylene glycol metabolism in animals involves oxidation by alcohol dehydrogenase to 2-hydroxyethoxy acetaldehyde which is further oxidized by aldehyde dehydrogenase to 2-hydroxyethoxyacetic acid (2-HEAA). The major part of absorbed diethylene glycol is excreted unchanged whereas the smaller part (< 37%) is oxidized to 2-HEAA. From the available data, metabolic breakdown of diethylene glycol to oxalate seems to be a minor route at most, indicating the stability of the ether linkage.

Diethylene glycol and its metabolites are readily cleared from the blood and excreted in the urine; 73% of ^{14}C-diethylene glycol activity was excreted with the urine after a single dose of 1.12 g/kg bw and 96% of ^{14}C-diethylene glycol activity in blood was excreted with the urine after a single dose of 11.2 g/kg. Only

small amounts are excreted via faeces (0.7-2.2%, after a single dose of 1.12 and 11.2 g/kg, respectively). For the first 6; 9; and 18 hours after oral administration of the 1.12; 5.6; and 11.2 g/kg bw doses, respectively, the urinary elimination rate of the ^{14}C label became constant, indicating zero-order elimination. Urinary elimination kinetics changed to first-order (with a half-life of 3 hours) at 6; 9; and 18 hours after oral doses of 1.12; 5.6; and 11.2 g/kg bw, respectively, indicating that diethylene glycol can accelerate its urinary elimination by its ability to induce osmotic diuresis. Some of the absorbed diethylene glycol is excreted as CO_2 (<7.2%; elimination of total ^{14}C in expired air). With increasing doses the portion of diethylene glycol which was oxidized to CO_2 decreased indicating saturation of metabolism at the higher doses.

Five days after single oral doses of 1.12; 5.6; and 11.2 g/kg bw ^{14}C-diethylene glycol, a total of 1.5-4.3% of radioactivity was found in tissues of rats.

Chapter 6

Mechanisms of action

As diethylene glycol is purified by fractionation from crude mixtures containing ethylene glycol, diethylene glycol in the fifties and sixties may have been contaminated with small amounts of ethylene glycol. As oxalate is the main metabolite of ethylene glycol in the body, such contamination may have led to the formation of oxalate from ethylene glycol and this may have biased the results of early studies. The purity of diethylene glycol is in some studies not mentioned and impurities like ethylene glycol have not been measured. As oxalate is a minor metabolite, if at all, of diethylene glycol it is possible that the oxalate-containing bladder stones and tumours found in early studies are (partly) attributable to the presence of ethylene glycol rather than to the presence of diethylene glycol.

Alternatively, the mechanism of diethylene glycol toxicity may parallel that of ethylene glycol. Metabolism of ethylene glycol yields oxalate ions which readily form calcium oxalate monohydrate crystals in the presence of calcium. Guo and Martin (2005) found that these crystals, and not the oxalate ions, are responsible for the membrane damage and subsequent cell death observed in normal human and rat proximal tubule cells.[35] Human case findings support the view of calcium oxalate crystals as the toxic agent of ethylene glycol poisoning.[36] Diethylene glycol may follow a similar toxic pathway if its major metabolite, 2-hydroxyethoxy acetate, precipitates in the presence of calcium.

The main health hazard of diethylene glycol is demonstrated by acute oral exposure. Experience in man following the administration of drugs formulated in

diethylene glycol has shown that ingestion of about 1 ml/kg bw can lead to severe intoxication, potentially fatal within a few days.[37] Death may occur from a single high dose or from repeated lower doses. Acutely toxic doses, not immediately fatal, exert their effect primarily on the kidney and, to a lesser extent, on the liver.[37-39] Renal insufficiency by swelling of the convoluted tubules and plugging of the tubules with debris may cause death or severe injury.[40] Chronic effects from prolonged and repeated exposure are likely to be centred in the kidney and to a lesser degree in the liver. Delayed onset of symptoms (vomiting 2 days post-ingestion and acute renal failure 6 days post-ingestion) has been reported in several fatalities.[40] Neurological defects have been observed several days after the exposure and included (partial) facial paresis, ascending paralysis and loss of sensory responses.[32,37,39,41-43] Electromyography revealed widespread acute denervation in arm and leg muscles, and brain MRI demonstrated enhancement of cranial nerves III and V.[43] Post-mortem analysis revealed severe demyelination and axonal damage of central and peripheral nerves.[41,42]

To determine whether the toxicity of diethylene glycol was due to its oxidation and/or its oxidation products, the effect of alcohol dehydrogenase inhibition by pyrazole on the lethality of diethylene glycol (single dose) was studied in male Wistar rats.[29] Pyrazole pretreatment reduced the lethality of diethylene glycol, indicating that the oxidation of diethylene glycol and/or its metabolite 2-HEAA contributed to the acute toxicity. Treatment of rats with bicarbonate for correction of metabolic acidosis and ethanol (inhibition of diethylene glycol metabolism by saturation of alcohol dehydrogenase) also prevented renal lesions and death.[44] According to Woolf[45], the analogy of diethylene glycol to other toxic glycols would dictate that an active hepatically produced metabolite of diethylene glycol is responsible for its toxic effects rather than the parent compound itself, although no information in humans is available as to what the putative toxin might be.

In man and animal, diethylene glycol produces a dose-dependent metabolic acidosis. In rats, the acidosis may resolve after 24-48 hours at doses up to 10 ml/kg bw (11.2 g/kg bw).[22] This finding was explained by the transient accumulation of 2-hydroxyethoxyacetic acid (2-HEAA) in the blood. At higher doses of diethylene glycol (>12.5 ml/kg bw; 13.9 g/kg bw), higher concentrations of 2-HEAA were produced which caused a collapse of the blood buffer systems. The metabolic acidosis can be further enhanced by accumulation of lactate. The highest lactate concentration in blood was found after 120 hours.[22] Metabolic acidosis in rats is known to inhibit hepatic gluconeogenesis *in vivo*, and therefore the concentration of lactate increased in these rats.

Diethylene glycol produces osmotic diuresis in laboratory animals. In rats, doses of 1 to 15 ml/kg bw (1.12 to 16.7 g/kg bw) produced a linear increase in the volume of urine excreted in 24 hours.[22] Oral doses of 19.5 g/kg caused a further increase of the urine volume in three out of ten animals, indicating limits to the renal excretion capacity. Doses of 16.7 and 19.5 g/kg diethylene glycol produced a 4-fold increase in the volume of the 24-hour urine as compared to that of control animals. Not all the rats were able to compensate the loss of 25% of body water by drinking because of the narcotic effect. After 24-48 hours, oliguria and anuria ensue. Rats subsequently developed symptoms of uraemia with unpleasant body odour, balance disorder, cold limbs, ruffled coat, turbid and pale red lens, tremors, spasms, and they died 1-2 days later in uraemic coma.

Chapter 7

Effects

7.1 Observations in humans

Available human data on the effects of diethylene glycol are summarised in Tables D-1 to D-5 of Annex D. The relevant studies are described in the following sections.

7.1.1 Irritation and sensitisation

Human data on skin and eye irritation and on sensitisation of diethylene glycol are summarised in Table D-2 of Annex D.

Skin irritation

A two-hour occlusive exposure, twice a day, of the forearm to undiluted diethylene glycol caused no irritation in humans.[46] In a Draize test with exposure to 112 mg diethylene glycol for 3 days, a slightly irritating reaction to human skin was observed.[2] In a patch test with 50 healthy volunteers, 20% was the highest concentration of diethylene glycol (in petrolatum) found to be non-irritant after 48-hour covered contact.[47] No skin reactions were reported in 480 dermatitis patients in a patch test (48 hours covered) with a 15% glycol mixture that contained unspecified proportions of diethylene glycol, ethylene glycol and propylene glycol in petrolatum.[47] Based on this limited number of available human studies, the

committee concluded that diethylene glycol does hardly if at all have any irritating effects to the human skin.

Eye irritation

No studies or case reports involving eye irritation in humans were available in literature.

Sensitisation

In a case report from 1938, a man developed an 'allergic dermatitis' (location on body not specified) 2-4 weeks after he had started smoking a brand of cigarettes containing diethylene glycol. In the patch test (24 hours covered), a 5% aqueous solution of diethylene glycol was found to cause allergic dermatitis.[48] No allergic reactions were reported in 480 dermatitis patients in a patch test (48 hours covered) with a 15% glycol mixture that contained unspecified proportions of diethylene glycol, ethylene glycol and propylene glycol in petrolatum.[47] The available data are not adequate to evaluate whether diethylene glycol has sensitising properties in humans.

7.1.2 Acute and short-term toxicity

Human accidents with regard to acute and short-term exposure to diethylene glycol are summarised in Table D-3 and D-4 of Annex D. No relevant acute inhalation data are available. Furthermore, no human data on short-term inhalatory diethylene glycol exposure are available.

The main reported health hazard of diethylene glycol is demonstrated by acute oral exposure.[37,45,49-56] The administration of drugs formulated in diethylene glycol showed that the ingestion of about 1 ml/kg bw (which is about 1 g/kg bw) can lead to fatal intoxication with multi-system organ failure. Renal failure is the most prominent cause of death. After large scale intoxication of Haitian children with a paracetamol syrup that contained diethylene glycol, it was estimated that a median dose of diethylene glycol of 1.34 ml/kg bw (1.49 g/kg bw; range: 0.22-4.42 ml/kg bw; 0.25-4.9 g/kg bw) caused acute renal failure.[37] The ingested dose in the Haiti accident was estimated by multiplying the percentage of diethylene glycol in the bottle – obtained from the child's parent – by the volume missing from the bottle. This method provided an estimate of the maximum possible dose ingested and the actual ingested dose could have been any amount less than the calculated dose. The authors also noted that the fatal and nonfatal dose ranges overlapped considerably.

Recently, Ferrari and Giannuzzi (2005)[57] estimated an acute lethal dose of 0.014 – 0.17 g/kg bw for humans from the analysis of the massive intoxication that occurred in Argentina in 1992. In the original paper, a calculation error was made and led to even 1000-fold lower dose estimates. The values reported by Ferrari and Gianuzzi (2005)[57] are the lowest values reported ever in fatal accidents. The estimation was based on the volumes of ingestion reported by family or relatives during interrogation. This way of estimating ingested doses is prone to error. The committee concluded that the dose estimates of O'brien *et al.* (1998)[37] are more reliable than those of Ferrari and Giannuzzi (2005).[57]

When comparing the median lethal dose of 1.49 g/kg bw with reported lethal doses for several animal species including rodents, the committee concludes that man is about 10 times more sensitive for the acute toxic effects of diethylene glycol than the animals used in toxicity studies (see Table 7.1).

Diethylene glycol poisoning in man is characterized by effects on the kidneys, liver, central nervous system, heart, pancreas, and the respiratory system. On presentation, clinical effects usually include vomiting, abdominal pain and pain in the kidney regions, fever, diarrhoea, headache, tachypnea, dehydration, hepatomegaly, splenomegaly, and jaundice.[58] Depending on the dose ingested, metabolic acidosis with Kussmaul respiration develops within one or two days after the ingestion. This may be followed by coma, convulsions and eventually death from acute renal failure. Pathological findings are hydropic degeneration of the kidney tubules and centrilobular areas of the liver, with generalised oedema and haemorrhages. Oxaluria or oxalate crystals have not been found in acute human diethylene glycol poisoning.[41-43,45,59] In two case reports, amorphous deposits of calcium containing material have been found in the tubules, again without crystal deposits.[60,61]

Acute and short-term neurological effects

Some of the human accidents with regard to acute and short-term exposure to diethylene glycol (see Table D-3 and D-4 of Annex D) describe neurological effects.

After accidental oral exposure to diethylene glycol, drowsiness, headache, impaired consciousness, progressive obtundation, coma and death haven been reported. Drowsiness may be delayed until approximately 24 hours after ingestion. In addition, meningism, cerebral oedema and haemorrhages, bulbar palsy, and (partial) facial and ascending paralysis have been reported.[32,37,40-43,60,61] occurring 1-3 weeks post ingestion. Electromyography revealed widespread acute denervation in arm and leg muscles, and brain MRI demonstrated enhancement

of cranial nerves III and V.[43] Pathologic examinations post mortem showed central and peripheral nervous system lesions, including axon damage followed by severe demyelination of virtually all cranial and peripheral nerves sampled, and sparing of central myelin.[41,42]

Neurological effects were also noted during severe intoxications after uptake of diethylene glycol in patients with burns. Five people with burns involving 7-62% of their body surface were treated with an 1% silver sulfadiazine formulation containing diethylene glycol and diethylene glycol stearate as a solvent (application to the skin for up to 24 days). Diethylene glycol and diethylene glycol stearate were present 'in a proportion of 6.2 to 7.1 g/kg of substance'. The patients developed acute anuric renal failure with metabolic acidosis and concomitant severe neurological abnormalities progressing to coma and finally death.[59]

7.1.3 Long-term toxicity

No human data on long-term diethylene glycol exposure are available.

Carcinogenicity

The International Agency for Research on Cancer has not evaluated diethylene glycol as a carcinogen.[62]

A Texas petrochemical plant had elevated standardized mortality ratios for neoplasms of the brain. A nested case-control study has examined possible associations between gliomas of the brain and job title, departmental employment history, chemical exposure history (including diethylene glycol), geographic location within plant, dates of employment and residence near the plant.[63] (see Table D-5 of Annex D). A total of 17 cases of gliomas were included in the study. Each case was matched with 6 controls. The greatest apparent risks were associated with exposure to carbon dioxide, diethyl sulphate, diethylene glycol, ethanol, ethylene, isopropanol, methane, tetraethylene glycol and vinyl acetate. Highest apparent risks were also associated with first employment in the 1940s or early 1950s, and with residence in the area around the plant. Unfortunately, the study lacked a quantitative estimate of diethylene glycol exposure and can, therefore, not be evaluated.

Reproduction toxicity (fertility and development)

No human studies or case reports with regard to reproduction toxicity were found in the literature.

Immunologic effects

No human studies or case reports on immunologic effects were found in the literature.

7.2 Animal experiments

7.2.1 Irritation and sensitisation

Animal data on skin and eye irritation and on sensitisation of diethylene glycol are summarised in Table E-1 of Annex E.

Skin irritation

Although none of the skin irritation studies were performed according to current guidelines for the testing of the health effects of chemicals as given by the Organisation for Economic Co-operation and Development (OECD), the skin irritation studies with rats, rabbits and guinea pigs showed that diethylene glycol did not or only slightly cause skin irritation (tested concentrations: 10-100% v/v diethylene glycol).[46,64]

In a repeated dose study, five female rabbits were exposed daily to a 0.5-ml mixture of equal parts of diethylene glycol and propylene glycol (semi-occlusive) for 100 days (exposure area 100 cm^2; no specification of exposure time per day; not specified whether the substance was removed after each day).[65] There were no macroscopic changes. Microscopic examination after 20-30 days showed a slightly thickened stratum granulosum and signs of proliferation in the stratum basale of the epidermis. Superficial portions of the dermis showed some infiltration with cells of the lymphatic series and histiocytes. The collagen fibres were slightly fragmented and scattered. The findings remained unaltered in later stages.

Eye irritation

In different studies with rabbits, rats, cats and dogs (see Annex E, Table E-1), not performed according to OECD-guidelines, undiluted diethylene glycol caused no eye irritation.[46,66] In another study, undiluted diethylene glycol was slightly irritating.[64] Based on these studies, diethylene glycol has minor or no eye-irritating properties at all.

Sensitisation

In a maximisation study with guinea pigs performed in accordance with Directive 84/449/EEC, B.6, diethylene glycol showed no sensitising properties (BASF, 1991 cited in DFG, 1995[67]; original study report not available; see Table E-1).

7.2.2 Acute toxicity

Acute toxicity studies are summarised in Table E-2 of Annex E.

Rats exposed for four hours to 4.4-4.6 g/m³ diethylene glycol aerosol (mass median aerodynamic diameter of the particles ranged from 2.6-3.1 µm; more than 96% of the particles were below 10 µm) developed decreased activity during exposure with rapid recovery on removal, a transient body weight loss with recovery in 3-5 days, and nasal discharge or lacrimation indicative for minor irritation which persisted for several days.[68] Because there were no deaths over the 14-day observation period, the 4-hour LC_{50} in rats was higher than 4.6 g/m³ (Table 7.1). Post-mortem examinations were unremarkable.

The acute oral toxicity of diethylene glycol is low in experimental animals (Table 7.1 and Table E-2 of Annex E). The oral LD_{50} values for mice and rats are in the range of 15-25 g/kg bw. For guinea pigs, oral LD_{50} values are in the range of 8-14 g/kg bw, and for rabbits, cats and dogs in the range of 4-11 g/kg bw. Rabbits, cats and dogs appeared to be more sensitive than mice and rats. Following oral administration of diethylene glycol, the clinical signs of toxicity are similar for mice, rats, rabbits, guinea pigs, hamsters, cats and dogs, and resemble those reported for humans. Acute toxic doses exert their effect on the central nervous system (dyspnoea), the kidney and, to a lesser extent, on the liver. Lethal doses cause renal failure with anuria, uraemic coma and death. Macroscopic and histopathological effects after a lethal dose include hydropic degeneration of the kidney tubules and the centrilobular areas of the liver, with generalized oedema and haemorrhages.

After a single oral dose (by gavage) of 1-17.5 ml/kg bw (1.12-19.5 g/kg bw) rats developed in the following order: narcotic phase, diuretic phase and thirst, drop of the pH of the urine and blood, and then, depending on dose, either recovery to normal or hydropic degeneration of the renal tubules, oliguria and anuria (which followed the polyuric phase 24-48 hours post-dosing), accumulation of urea and uric acid in the blood and, finally, death after 2-7 days (urine production ceased), from non-compensated metabolic acidosis and uraemia. Twenty-four hours after oral doses of 16.7 g/kg diethylene glycol, one out of ten rats developed anuria, and 48 hours after oral doses of 19.5 g/kg diethylene glycol, five out of ten rats became anuric and developed symptoms of uraemia with disorders of balance, tremors, spasm, and an unpleasant body odour, cold limbs, ruffled coat, turbid and pale red lens, and they died 1-2 days later in uraemic coma. However, death caused by doses of 19.5 g/kg bw diethylene glycol also occurred in the acute phase of intoxication, *i.e.*, within 24 h, presumably by a direct damaging effect. When the dose was increased from 1.12 g/kg bw to 19.6 g/kg bw, the narcotic and diuretic effects increased.[22,30]

In a study which was focussed on renal impairment, SD rats were exposed once by gavage to 0.2, 0.7, 2.0 or 8.0 g/kg bw (n=6 females/dose).[78] No adverse effects were observed in animals exposed to 0.2 g/kg bw. In urine of rats treated with 0.7 g/kg bw diethylene glycol, lactate dehydrogenase activity was significantly enhanced one day after treatment. After 2.0 g/kg bw, an additional rise in urinary beta-galactosidase activity two days after treatment, and a significant rise of urinary volume and a decrease of urine creatinine and pH on the first day after exposure. In addition to the changes mentioned above, after one day, leucine aminopeptidase activity in the urine was significantly elevated at 8.0 g/kg bw and the specific gravity of the urine was decreased. However, in all animals the wet weight of the kidneys remained normal as compared to controls. The results thus show transient dose-dependent changes in several renal parameters, indicating a slight-to-moderate and reversible renal impairment.

LD_{50} values after dermal exposure to diethylene glycol in rats and rabbits were 13-20 g/kg bw (see Table E-2 of Annex E).

Table 7.1 Acute toxicity of diethylene glycol.[69,70]

Species	Route of administration	LD_{50} (ml/kg bw)[a]	(g/kg bw)	Lethal dose (ml/kg bw)	(g/kg bw)	Reference
Mouse	Oral	23.7	26.4			Laug *et al.* (1939)[71]
Mouse	Oral	25.2	28.1			Loeser *et al.* (1954)[46]
Mouse	Oral		13.3			Plugin (1968) cited in Rowe and Wolf (1982)[72]
Mouse	Subcutaneous	20.2	22.5			Loeser *et al.* (1954)[46]
Mouse	Subcutaneous			5	5.6	Oettingen and Jirouch (1931) cited in Browning (1965)[73]
Mouse	Intraperitoneal		9.7			Karel *et al.* (1947)[74]
Rat	Oral	14.8	16.5			Laug *et al.* (1939)[71]
Rat	Oral	25.7	28.7			Loeser *et al.* (1954)[46]
Rat	Oral		20.8			Smyth *et al.* (1941)[75]
Rat	Oral		15.6			Plugin (1968) cited in Rowe and Wolf (1982)[72]
Rat	Oral			15.0[b]	16.7[b]	Haag and Ambrose (1937)[76]
Rat	Oral	27.0	30.1			Weatherby and Williams (1939)[77]
Rat	Subcutaneous	18.1	20.2			Loeser *et al.* (1954)[46]
Rat	Subcutaneous			15.0[b]	16.7[b]	Haag and Ambrose (1937)[76]
Rat	Subcutaneous	16.8	18.7			Union Carbide Co cited in Rowe and Wolf (1982)[72]
Rat	Intramuscular			7.0[b]	7.8[b]	Haag and Ambrose (1937)[76]
Rat	Intravenous			5.0[b]	5.6[b]	Haag and Ambrose (1937)[76]
Rat	Intravenous	5.8	6.5			Weatherby and Williams (1939)[77]
Rat	Intravenous	8.0	8.9			Union Carbide Co cited in Rowe and Wolf (1982)[72]
Rat	Intraperitoneal	6.9	7.6			Union Carbide Co cited in Rowe and Wolf (1982)[72]
Guinea pig	Oral	7.8	8.7			Laug *et al.* (1939)[71]
Guinea pig	Oral		13.2			Smyth *et al.* (1941)[75]
Guinea pig	Oral		14.0			Plugin (1968) cited in Rowe and Wolf (1982)[72]
Rabbit	Oral		26.9			Plugin (1968) cited in Rowe and Wolf (1982)[72]
Rabbit	Intravenous	1-2	1.1-2.2			Kesten *et al.* (1937) cited in Browning (1965)[73]
Rabbit	Intravenous			2.0[b]	2.2[b]	Haag and Ambrose (1937)[76]
Rabbit	Intramuscular			4.0[b]	4.5[b]	Haag and Ambrose (1937)[76]
Rabbit	dermal	11.9	13.3			Union Carbide Co cited in Rowe and Wolf (1982)[72]
		LC_{50} (g/m^3)				
Rat	4-hour aerosol inhalation	> 4.6				Cascieri *et al.* (1991)[68]
Mouse	2-hour inhalation	> 0.13				Cited by NIOSH[70]

a LD_{50}, lethal dose for 50% of the exposed animals; LC_{50}, lethal concentration for 50% of the exposed animals
b minimum lethal dose that kills 60% of the animals tested (Haag and Ambrose, 1937)[76]

7.2.3 *Short-term toxicity*

Short-term toxicity studies are summarised in Table E-3 of Annex E.

With regard to oral exposure many studies were cited in different review articles. More recent studies were performed by Huber *et al*. (1986)[79], Rossa and Weber (1987)[80], Ogbuihi *et al*. (1991)[81], Freundt and Weis (1989)[78] and Williams *et al.* (1990)[20]. Yet, the animal database on the effects of short-term exposure is limited, because several studies focussed on specific effects of diethylene glycol. Besides, in earlier studies significant amounts of ethylene glycol may have been present in the diethylene glycol. In the more recent studies, diethylene glycol exposure has been shown to result in tremors, lethargy and piloerection[20], decreased renal function[78], retinal damage (histopathological as well as electrophysiological)[80], increased serum glutamic oxaloacetic aminotransferase activity[79], prolonged blood coagulation time and decreased antibody response[79], myocardium damage (microscopical and ultrastructural)[81], and in mortality[80]. The study by Huber et al. (1986)[79] reported the lowest LOAEL, but this study can not be evaluated properly because the mice were also immunised with tetanus toxoid, Vaccinia virus and human erythrocytes, one week after start of the experiment.

With regard to dermal exposure to diethylene glycol, five female rabbits were exposed daily to a 0.5-ml mixture of equal parts of diethylene glycol and propylene glycol (semi-occlusive) for 100 days (see also section 7.2.1 Skin irritation).[65] From the study description, no data are available on systemic effects.

7.2.4 *Long-term toxicity*

Animal data on long-term toxicity of diethylene glycol are summarised in Table E-4 of Annex E.

Two inhalation studies in Russian were available in the literature. Different review articles concluded that an evaluation of these studies was not possible because of insufficient description of test set-up and results.[48,67,69]

Gaunt *et al.* (1976) performed a long-term study in rats.[1] Groups of 15 rats of each sex were given diets containing 0%, 0.4%, 2.0% and 4.0% diethylene glycol (equivalent to 0; 300; 1,550; and 2,970 mg/kg bw/day in the male rats; and 0; 360; 1,810; and 3,680 mg/kg bw/day in the female rats) for 14 weeks. In a second experiment, groups of ten rats of each sex were given diets containing 0%, 0.085%, 0.17%, 0.4% and 2.0% of the same sample of diethylene glycol (equivalent to 0; 51; 105; 234; and 1,190 mg/kg bw in the male rats; and 0; 64; 126; 292;

and 1,460 mg/kg bw/day in the female rats) for 225 days. The diethylene glycol used contained less than 0.01% (w/w) ethylene glycol and 0.03% (w/w) triethylene glycol. A level of 2,970 mg/kg bw/day caused the death of six male rats with signs of renal damage. At autopsy, the rats had enlarged kidneys with distended pelvic area and pale cortical tissue. Cortical cysts were seen in two of these animals. On histological examination, tubular necrosis, mainly of the proximal convoluted tubules, was evident. The bladders and urethers were distended and the bladders contained 'oxalate' crystals, identified by light microscopy, and red blood cells. The surviving rats showed a reduced growth rate, increased water intake, increased urinary flow, signs of haemoconcentration, enlarged kidneys with hydropic degeneration of the tubular cells and with tubular necrosis. Liver pathology (hydropic degeneration and centrilobular necrosis) was seen in the females at the highest dose level of 3,680 mg/kg bw/day.

In the 14-week study, dietary concentrations of 360 mg/kg bw/day and higher resulted in urinary 'oxalate' crystals in the females, and at the highest dose level in both males and females (Table 7.2). Hydropic degeneration of the kidneys (and also of the liver in the 4%-dosed females), tubular necrosis in the kidneys, and increased urine volumes after concentration tests (see legend to Table 7.2) were seen at the highest dose levels of 2,970 mg/kg bw/day in the males and 3,680 mg/kg bw/day in the females. One of the 15 males already developed hydropic degeneration of the kidneys at 1,550 mg/kg bw/day (2% diethylene glycol in food).

In the 225-day study, the only finding at a dietary level of 105 mg/kg bw/day was a 13-23% increase of male urinary 'oxalate', measured by the colourimetric assay of Hodgkinson and Williams (1972)[82], whilst at the lowest level (51 mg/kg bw/day) no effects were observed. Urinary 'oxalate' crystals were found in the males at the dose level of 234 mg/kg bw/day only, and in the females at dose levels starting from 292 mg/kg bw/day. In contrast, the number of rats with phosphate crystals decreased with increasing diethylene glycol intake. Increased urine volumes after concentration tests were seen in the males only, occurring from dose levels of 234 mg/kg bw/day. Histological examination of the kidneys did not reveal any renal damage at any dose level (0-2% diethylene glycol) in the 225-day study. The data are summarized in Table 7.2.

The oxalate crystals found in this study were not positively identified and urinary oxalate concentrations may have been overestimated in the colourimetric assay used by the authors. In view of oxalate being a minor metabolite of diethylene glycol at best, it is the committee's opinion that the oxalate crystals reported by

Gaunt *et al.* (1976)[1] are based on a mistaken identity, and may have been or may have included 2-HEAA.

From the results of the study by Gaunt *et al.* (1976)[1], the committee considered the effects on the kidneys as the critical effects of diethylene glycol. The effects include 'oxalate' crystaluria, increased urine volumes after concentration tests, and the histopathological findings of hydropic degeneration and tubular necrosis. For 'oxalate' crystaluria and increased urine volumes after concentration tests, the results were inconsistent between the male and the female rats, and no clear dose-response relationships can be observed for these effects. For example, the number of male rats with urinary 'oxalate' crystals was not increased at the highest dose level of 1,190 mg/kg bw/day in the 225-day study.

Table 7.2 Urinary 'oxalate' crystals, urine volumes after water deprivation/load, and kidney damage in Wistar rats given 0-4% diethylene glycol in food for 14 weeks and 0-2% diethylene glycol in food for 225 days, respectively.[1]

Diethylene glycol in food (%)	Mean diethylene glycol intake (g/kg bw/day)		Number of rats with urinary 'oxalate' crystals		Urine volume (ml) after concentration test[a]				Number of rats with hydropic degeneration of the kidneys or tubular necrosis	
					0-6 hours		16-20 hours			
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
14 weeks										
0	0	0	0/15	0/15	3.0	1.5	0.2	0.1	0/15	0/15
0.4	0.30	0.36	0/15	7/15**	2.8	1.7	0.1	0.2	0/15	0/15
2.0	1.55	1.81	3/15	7/15**	2.3	2.3	0.2	0.2	1/15	0/15
4.0	2.97	3.68	6/8***	13/15***	4.7[b, *]	2.1	0.6[b, *]	0.5[b, **,]	6/9*	9/15***
225 days										
0	0	0	1/10	1/10	2.4	1.7	0.6	0.6	0/10	0/10
0.085	0.051	0.064	3/10	1/10	1.9	0.9	0.9	0.6	0/10	0/10
0.17	0.105	0.126	3/10	2/10	1.8	1.7	1.0	0.8	0/10	0/10
0.40	0.234	0.292	6/10	4/10*	2.1	1.7	1.1	0.7	0/10	0/10
2.0	1.19	1.46	2/10	6/10	2.6	2.0	1.3	0.7	0/10	0/10

[a] Urine volume measured during 0-6 hours of water deprivation and during 16-20 hours after a 25 ml/kg bw water load. Values are means for the number of rats in each group.

[b] Mean urinary specific gravity in this group of rats was significantly decreased compared to controls.

Statistically significantly different from controls: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

In addition, the observed increase of urinary volumes may partly have resulted from the osmotic-diuretic effect of diethylene glycol and the 'oxalate' crystaluria can not be explained in view of later findings that oxalate is no or at best a minor metabolite of diethylene glycol in rats. The relevance of the 'oxalate' crystals found by Gaunt *et al.* (1976) is therefore unclear. For these considerations, the committee concluded that the NOAEL based on the histopathological findings is more relevant than the NOAEL based on the urinary 'oxalate' crystals and the increased urine volumes after concentration tests.

NOAEL's and LOAEL's for the different renal effects of diethylene glycol as described by Gaunt *et al.* (1976)[1] are given in Table 7.3. The committee noted that in the 14-week study hydropic degeneration was found in one of the male rats at the dose level of 1,550 mg/kg bw/day (2% diethylene glycol in food). From this, the committee concluded that histopathological effects started to occur at 1,550 mg/kg bw/day and that the NOAEL for hydropic degeneration is 300 mg/kg bw/day (0.4% diethylene glycol in food) in the male rats.

Table 7.3 NOAEL's and LOAEL's for the renal effects of dietary diethylene glycol in Wistar rats.[1]

Physiological effect	NOAEL % diethylene glycol (g/kg bw/day)		LOAEL % diethylene glycol (g/kg bw/day)	
	Males	Females	Males	Females
14 weeks of exposure				
Urinary 'oxalate' crystals	2.0 (1.55)	< 0.4 (< 0.36)	4.0 (2.97)	0.4 (0.36)
Urine volume[a]	2.0 (1.55)	2.0 (1.81)	4.0 (2.97)	4.0 (3.68)
Histopathological effect (kidney)				
Hydropic degeneration	0.4 (0.30)	2.0 (1.81)	2.0 (1.55)[b]	4.0 (3.68)
225 days of exposure				
Urinary 'oxalate' crystals	0.17 (0.105)	0.17 (0.126)	0.4 (0.234)	0.4 (0.292)
Urine volume[a]	0.17 (0.105)	> 2.0 (> 1.46)	0.4 (0.234)	> 2.0 (> 1.46)
Histopathological effect (kidney)				
Hydropic degeneration	> 2.0 (> 1.19)	> 2.0 (> 1.46)	> 2.0 (> 1.19)	> 2.0 (> 1.46)

[a] Urine volume measured 16-20 hours after a 25 ml/kg bw water load.
[b] One of 15 males had hydropic degeneration of the kidneys and this is considered relevant by the committee.

Long-term oral studies before 1950 revealed renal effects, bladder stones, and bladder tumours in rats given diethylene glycol in food. In a number of these studies bladder tumours have been associated with the formation of oxalate-containing bladder stones.[83] Weil *et al.* (1965; 1967)[84,85] found that diethylene glycol did not induce bladder tumours in rats unless a foreign body or lesion was present, such as an oxalate-containing bladder stone or a surgery-induced bladder lesion. These authors concluded that the bladder tumours they found in their chronic feeding experiment with diethylene glycol were the result of mechanical irritation by oxalate-containing bladder stones and that the low tumourogenic response of diethylene glycol in their studies – as compared to previous studies – resulted from the relative purity of diethylene glycol that had become essentially

free of ethylene glycol (which is metabolised to oxalate). No information was found in the literature concerning the occurrence of bladder stones in humans after ingestion of diethylene glycol.

In a carcinogenicity study, Fischer 344 rats were exposed orally to 0; 1,200; and 2,600 mg/kg bw/day diethylene glycol (purity 97%, ethylene glycol content was not stated) for 108 weeks.[19] At the dose level of 2600 mg/kg bw/day, increased drinking water consumption (females 17% and males 25%) and mortality of 19/50 males (compared to 13/50 in the control group) occurred. The serum lactate dehydrogenase activity was increased and serum urea nitrogen was decreased in males, the creatinine phosphokinase activity and the absolute and relative weights of the lungs were increased in both sexes (all of these changes were significant). In the urine no changes could be detected, but it was not mentioned if oxalate was measured. No bladder stones were reported and hyperplasia of the urinary tract epithelium was not observed. Only one kidney carcinoma was detected (1/100). At the lower dose one kidney carcinoma and one nephroblastoma were recorded.

No dermal long-term exposure studies were available.

Mutagenicity and genotoxicity in vitro

In Annex E, Table E-5, the available data with regard to *in vitro* genotoxicity are summarised.

In the Ames test, diethylene glycol was not mutagenic in Salmonella typhimurium strains TA97a, TA98, TA100, TA102, TA1535, TA1537 and TA1538 with and without metabolic activation in concentrations up to 100 mg/plate.[86-90] A weak mutagenic effect could be detected in strain TA104 in the presence of metabolic activation (maximum: 2.2 fold increase over the spontaneous reversion frequency at 315 µmol diethylene glycol /plate).[90] Furthermore, diethylene glycol (150 and 750 µmol/ml) was negative in a reverse mutation test with Saccharomyces cerevisiae D7 and D61M.[91] In the absence of metabolic activation, an increase of the mitotic aneuploidy rate was observed in an aneuploidy test with Saccharomyces cerevisiae D61M.[90] Negative results in the absence and presence of a metabolic activation were observed in a chromosome aberration test with Chinese hamster ovary cells, in a HPRT-test with Chinese hamster ovary cells, in a SCE-test with Chinese hamster ovary cells (all three test were performed with concentrations up to 50 mg/ml) and in a SOS chromotest with Escherichia coli PQ37.[89,92]

Taken together, the available data indicate that diethylene glycol is not genotoxic *in vitro*.

Mutagenicity and genotoxicity in vivo

In Annex E, Table E-6, the available data with regard to *in vivo* genotoxicity are described.

Diethylene glycol was tested *in vivo* on its potency to induce chromosome aberrations in Chinese hamsters (Japanese paper, only tabulated data in English).[88] Exposure was by intraperitoneal injection, by oral dose, exposure via drinking water or via the diet. A number of 100 cells was analysed for all concentrations in the various exposure scenarios. The background in the controls was one or two aberrations in 100 analysed cells for all scenarios, regardless the treatment time. Slight increases in the number of chromosome aberrations were found, but because the authors did not differentiate between chromosomal gaps and chromosomal breaks, the results are difficult to interprete. Intraperitoneal injection and oral dosing included treatment times of 6; 24; or 48 hours at doses between 312.5 and 7,500 mg/kg bw. Slight increases in the incidence of chromosome aberrations were found after intraperitoneal injection of 1,250 mg/kg (3, 6 and 3 aberrations after 6; 24; and 48 hours); 2,500 mg/kg (4, 7 and 2 aberrations after 6; 24; and 48 hours) and 5,000 mg/kg (7, 5 and 7 aberrations after 6; 24; and 48 hours), respectively (7,500 mg/kg was not done). Oral dosing induced chromosome aberrations only at 7,500 mg/kg (4, 3 and 4 aberrations after 6, 24 and 48 hours, respectively). Exposure via the drinking water at levels between 0.5% and 2.0% for 1, 2 or 3 weeks resulted in an increase of aberrations at all dose levels. At the 0.5% dose level, the highest number of aberrations (4) was observed after 2 weeks of exposure. At 1.0 and 2.0% the most pronounced effects were 4 aberrations (after 2 weeks) and 6 aberrations (after 1 week), respectively. After exposure via the diet for a period up to 12 weeks at dose levels between 1.25% and 5%, the number of chromosome aberrations was similar to the control (1-2 aberrations per 100 cells).

Diethylene glycol induced chromosome aberrations in a micronucleus test (species not reported) after a single intraperitoneal injection of 60% of the LD_{50} (not further specified, only abstract available) which causes organ damage such as tubular necrosis. This induction was suppressed when the animals were pretreated during 7 days with a low daily dose of diethylene glycol (4% of the LD_{50}).[91] The data given are insufficient to evaluate the genotoxicity of diethylene glycol *in vivo*.

BIBRA (1993)[48] and the IUCLID dataset 2000[2] cited a Russian study of Barilyak (1985)[93] in which an increase in chromosome damage in the bone marrow cells was observed in hamsters after administration by gavage of 1/5 of the LD_{50}.

Also, dominant lethal mutations were observed in rats after administration of diethylene glycol (Barilyak 1985[93] cited in BIBRA 1993[48]).

Overall, weak genotoxic effects *in vivo* are seen only at high doses of diethylene glycol. The committee does not consider this as an indication of genotoxicity.

Carcinogenicity

Carcinogenicity studies of diethylene glycol are summarised in Table E-7 of Annex E. Different results have been obtained probably related to contamination of the test substance.

The International Agency for Research on Cancer has not evaluated diethylene glycol.[62]

Long-term oral studies from before 1950 are difficult to interpret due to possible contamination of diethylene glycol with ethylene glycol which is metabolised into oxalate. These studies revealed renal effects, bladder stones, and bladder tumours. In a number of these studies bladder tumours are reported in connection with formation of oxalate-containing bladder stones. More recent studies demonstrated that in rats exposed to diethylene glycol, bladder tumours never developed without the preceding or concurrent presence of a foreign body such as an oxalate-containing bladder stone (see section 7.2.4).

More recent studies of Masui *et al.* (1988)[94] (diethylene glycol purity not stated), Ito *et al.* (1988)[95] (no information available on diethylene glycol purity) and Hiasa *et al.* (1990)[19] (diethylene glycol purity 97%, ethylene glycol content was not stated) and Hiasa (1991)[96] showed that diethylene glycol lacks carcinogenic properties after oral administration.

The studies of Dontenwill *et al.* (1970)[97] and Dunkelberg (1987)[25] showed that diethylene glycol (no information available with regard to diethylene glycol purity) is also lacking carcinogenic properties after dermal administration.

Reproduction toxicity

Animal data on reproduction toxicity of diethylene glycol are summarised in Table E-8 of Annex E.

Fertility

No suitable inhalation or dermal studies with regard to effects of diethylene glycol on fertility were available.

In an oral two-generation study employing a continuous breeding protocol with mice (Swiss CD-1), F0 mice were exposed to drinking water containing 0.0, 0.35%, 1.75%, and 3.5% diethylene glycol (purity > 99%) (n=20 pairs/dose).[20,98] Based on water consumption data collected during the study, these concentrations produced calculated diethylene glycol consumptions of about 610; 3,100; and 6,100 mg/kg bw/day. While F0 body weight was unchanged by diethylene glycol consumption during the mating period, the number of litters per pair was reduced by 12% at the top dose, and the number of live pups/litter was reduced by 32%. Furthermore, a significant increase in the cumulative days to litter and a significant decrease in the number of pairs producing the third, fourth, and fifth litters (increase in the number of infertile pairs) was observed at the top dose. In a crossover mating to determine the affected sex, there were no significant effects on mating index and fertility and the number of pups/litter was equivalent across the three groups. For the F1 mating trial, exposed mice from the 3,100 mg/kg bw/day group were used, because insufficient mice were available from the top dose, due to mortality and reduced fertility in that group. After all the F2 litters were born and the F1 females subjected to oestrous cyclicity evaluation, the F1 mice were killed and necropsied. Sperm indices (concentration and percentage of motile or abnormal sperm in the cauda epididymis) in the males exposed to 3,100 mg/kg bw/day were unchanged. In summary, diethylene glycol at 6,100 mg/kg bw/day was a reproductive toxicant in Swiss mice, based on reductions in litters/pair, and in mean litter size. In F0 mice, slight maternal (F0) toxicity was noted for this highest dose group (7% decrease in body weight). In F1 mice, at the highest dose, no adverse effects on fertility were observed.

Developmental toxicity

In the oral two-generation study by Williams *et al.* (1990) who used a continuous breeding protocol with Swiss CD-1 mice (see section fertility), F0 mice were exposed to drinking water containing 0; 610; 3,100; and 6,100 mg/kg bw/day diethylene glycol (n=20 pairs/dose).[20] Pup weight adjusted for litter size was reduced by 12% at the top dose level. The fifth or final litters from the 6,100 mg/kg bw/day dose group consisted of fewer live pups and the pups had significantly depressed birth weights. For these litters, twelve percent of the liveborn pups and 95% of the pups found dead on postnatal day 0 had craniofacial malformations including exencephaly and cleft palate. By the second day after birth, 50% of the malformed pups had died. Similar malformations were also noted for live and dead pups in the other litters exposed to 6,100 mg/kg bw/day diethylene glycol. In the cross-over mating experiment, adjusted pup weight was reduced by

9% in the control males x 6,100 mg/kg bw/day diethylene glycol females mating. After the F1 mice were weaned, the control and 6,100mg-dosed F0 mice were killed and necropsied. There were no treatment-related changes in male body or organ weights or histopathology, while female body weight was reduced by 7% after 6,100 mg/kg bw/day diethylene glycol consumption. Adjusted organ weights were unchanged. In the F1 mating trial, diethylene glycol at 3,100 mg/kg bw/day did not affect pup survival to mating at postnatal day 74. There were no treatment-related alterations in the number or weight of F2 pups in this mating trial. Necropsy of F1 mice revealed an 11% and 7% decrease in the body weights of the treated males and females, respectively. Absolute organ weights were not affected for both sexes. In summary, F1 mice exposed to 6,100 mg/kg bw/day had decreased body weights at birth and poor postnatal survival. At the intermediate dose of 3,100 mg/kg bw/day, F1 body weights of both sexes were depressed at weaning, at onset of mating, and at necropsy.

In another study, diethylene glycol (purity > 99%, ethylene glycol content < 0.2%) was administered by gavage to timed-pregnant Swiss (CD-1) mice (26-31 per group) on gestational days (GD) 6-15 at dose levels of 0; 1,250; 5,000; or 10,000 mg/kg body weight/day.[99,100] Animals were observed daily for clinical signs of toxicity. Food and water consumption and body weights were determined on GD 0, 3, 6, 9, 12, 15, and 17. All animals were killed on GD 17 and examined for maternal body and organ weights, implant status, foetal weight, sex, and morphological development. Maternal body weights did not differ significantly between the control group and any of the diethylene glycol-treated groups. Relative (g/kg body weight/day) water intake was significantly increased over control for every interval starting at GD 6 in the 5,000 and 10,000 mg/kg bw/day diethylene glycol treated animals. Maternal animals given 10,000 mg/kg bw/day of diethylene glycol had significantly decreased relative (g/kg body weight/day) food consumption from GD 6 to 12. One maternal animal treated with 10,000 mg/kg bw/day of diethylene glycol was sacrificed in extremis on GD 10. Necropsy and histopathologic examinations revealed evidence of renal degeneration and suggested that morbidity was due to toxicity produced by diethylene glycol. Necropsy of maternal animals on GD 17 showed that animals from the 5,000 and 10,000 mg/kg bw/day diethylene glycol groups had significantly increased absolute and relative kidney weights when compared to control animals. At 10,000 mg/kg bw/day, renal lesions (renal tubular degeneration) were noted in 2/27 females which survived to scheduled death. At the high dose, 11% (3/28) of the pregnant females showed evidence of renal pathology as compared to 0/20 pregnant females from the vehicle control group. No effects of diethylene glycol were observed on pre- or post-implantation loss. The mean foe-

tal body weight per dose group on GD 17 was associated with a significant decreasing linear trend (99%, 96%, and 85% of control from the low to high dose) and mean foetal body weight was significantly decreased in the high dose group (0.865 g) when compared to controls (1.012 g). Examination of the foetuses for external, visceral and skeletal malformations did not reveal any significant effects between dose groups. The decrease in foetal body weight indicated developmental toxicity at the 10,000 mg/kg bw/day exposure level of diethylene glycol. In summary, there was no maternal or developmental toxicity at 1,250 mg/kg bw/day of diethylene glycol. The mid-dose (5,000 mg/kg bw/day diethylene glycol) produced significant maternal toxicity, but no clear evidence of developmental toxicity. The high dose (10,000 mg/kg bw/day diethylene glycol) caused the death of 1 out of 28 pregnant dams, maternal toxicity and developmental toxicity.

In CD rats exposed to 0; 1.0; 4.0; and 8.0 ml/kg bw/day (respectively 0; 1,115; 4,460 and 8,920 mg/kg bw/day) diethylene glycol (purity unknown) by gavage (n=25/dose) during gestation days 6-15, developmental delay was observed at 4,460 and 8,920 mg/kg bw/day only in the presence of maternal effects.[101] Three dams at 8,920 mg/kg bw/day died on gestation day 11. Maternal effects observed in surviving dams at 8,920 mg/kg bw/day included reduced gestational body weight and food consumption, and increased water consumption and kidney weights. Maternal kidneys showed interstitial nephritis and tubular basophilia indicative of renal tubule damage and repair. At 4,460 mg/kg bw/day, increased water consumption, reduced food consumption and decreased corrected weight gain were observed. No foetal malformations were observed at any dose level. Litter weights were reduced and the incidences of five skeletal variations were increased in foetuses at 8,920 mg/kg bw/day. One skeletal variation was increased at 4,460 mg/kg bw/day. Thus, minor developmental effects, developmental delay, reduced litter weight and (an) increase(s) in incidence(s) of skeletal variation(s) were seen in the presence of maternal effects from 4,460 mg/kg bw/day. No foetal malformations were observed at any dose level.

Timed-pregnant CD-1 mice (n=30) and CD rats (n=25) were dosed daily by gavage with undiluted diethylene glycol over gestational days 6-15.[102] Mice received 0 (distilled water); 559; 2,795 and 11,180 mg/kg bw/day, and rats 0; 1,118; 4,472; and 8,944 mg/kg bw/day. They were examined daily for clinical signs of toxicity. Body weights, food consumption and water consumption were measured periodically throughout gestation. At necropsy, on gestational day 18 (mice) or 21 (rats), dams were examined for gross pathology, and bode, gravid uturus, liver and kidney weights were measured. Six of 30 female mice dosed with 11,180 mg/kw bw/day died from gestational day 7-10, that is after 1-5 days

of dosing with undiluted diethylene glycol. There were no mortalities in the mid and low dosage groups. The cause of death was unknown, and the clinical signs (seen at 11,180 mg/kg bw/day only) included hypoactivity, prostration, labored and slowed breathing, and cold extremities. Body weights and body weight changes were similar across all groups. Statistically significant increases in water consumption were found at the dose level of 11,180 mg/kg bw/day over gestational days 6-9, 9-12, and 12-15; and at 2,795 mg/kg bw/day over gestational days 6-9, 9-12, and 12-15. Implantations were comparable across all groups. Fetal body weights were significantly reduced at 11,180 mg/kg bw/day without increases in variations or malformations, either total, by category, or individually. With rats, maternal toxicity was present at 8,944 mg/kg bw/day (3 females died on gestational day 11, clinical signs, reduced body weight gain, reduced food consumption, increased water consumption, increased liver weight, increased kidney weight, and renal histopathology) and at 4,472 mg/kg bw/day (increased water consumption). Fetal body weights were significantly reduced at 8,944 mg/kg bw/day. There were no significant effects with respect to total or individual external or visceral variations. Individual skeletal variations were significantly increased at 8,944 mg/kg bw/day and at 4,472 mg/kg bw/day, consistent with reduced fetal body weight. Malformations (total, by category or individual) were similar for all groups. For maternal toxicity, the no-observed-effect-level for diethylene glycol given by gavage over gestational days 6-15 was 559 mg/kg bw/day with the mouse and 1,118 mg/kg bw/day with the rat. For developmental toxicity, the no-observed-effect-levels were 2,795 mg/kg bw/day with mice and 1,118 mg/kg bw/day with rats.[102]

No dermal study with regard to developmental effects of diethylene glycol was available.

Immunological effects

NMRI mice (n=20/dose) were exposed to 0%; 0.03% (50 mg/kg bw/day); 0.3% (500 mg/kg/day); and 3% (5,000 mg/kg/day) diethylene glycol in drinking water for 4 months (14-17 weeks; see Table E-3 of Annex E).[79] One week after start of the experiment, animals were immunised with tetanus toxoid, Vaccinia virus and human erythrocytes. After 3.5 months, the two higher dose groups had a statistically significant (50%) reduction in serum titre of tetanus antibodies, indicating reduced immune defence, although a clear dose-response relationship was lacking. No effects were seen on the delayed type hypersensitivity reaction towards tetanus toxoid and human erythrocytes. After 4 months exposure, all the mice were inoculated with *Streptococcus pyogenes.* Exposure to 500 mg/kg/day dieth-

ylene glycol significantly enhanced streptococcus induced mortality in mice. At 5,000 mg/kg/day, streptococcus induced mortality in mice was not increased. Because of the absence of a dose-relationship, the relevance of the observed immunological effects is not clear.

Neurological effects

After acute and short-term inhalation, oral and dermal exposure to diethylene glycol different neurological effects were observed (see Table E-2 and Table E-3 of Annex E).

Besides other effects, excited behaviour and general anaesthetic response were observed in mice exposed by inhalation for 2 hours to 130 mg/m^3 (Sanina and Kocketkova, 1966 (article in Russian) evaluated by BIBRA (1993)[48] and by the Nordic steering group for assessment of health effects of chemicals (1998)[17]). Rats showed decreased activity during exposure to 4.4-4.6 g/m^3 with rapid recovery after discontinuation of exposure.[68]

Ataxia, lethargy and dyspnea were observed in rats given a single dose of 6 or 12 ml/kg bw (6.7 or 13.4 g/kg bw) by orogastric intubation.[21] The animals regained normal activity within 6 to 8 hour post-dosing. Eight of the 24 animals exposed to 24 ml/kg bw (26.8 g/kg bw) died within 48 hours. Four of these were active and alert at 36 hours post-dosing. All of the animals exposed to 26.8 g/kg bw were alert and active for at least 6 hour post-dosing.[21]

After single oral administration of 1-17.5 ml/kg bw (1.12-19.5 g/kg bw), rats developed a narcotic phase. From the study descriptions, it was not clear from which dose this effect occurred.[22,30]

Drunken gait, general lassitude, coma, muscular tremors, spasms and death were observed in dogs exposed to a total dose 9 ml/kg bw (10 g/kg bw).[103] At a single intraperitoneal dose of 11 g/kg bw, convulsions and death were observed in rats.[104]

7.3 Summary and evaluation

Human data

Based on the limited number of human studies available, diethylene glycol has no or only mild irritating effects to the human skin. No human data on eye irritating properties of diethylene glycol were found. Adequate data to evaluate whether diethylene glycol has sensitising properties in humans were absent.

In humans, the main health hazard of diethylene glycol is demonstrated by acute oral exposure. The administration of drugs formulated in diethylene glycol has shown that ingestion of about 0,5-1 g/kg bw can lead to severe intoxication with fatal consequences due to renal failure. Initial effects of diethylene glycol poisoning include dizziness, vomiting, headache, polyuria, and abdominal and back pains. Within one or two days, metabolic acidosis develops and renal function deteriorates. If untreated, this may proceed to fulminant renal failure with coma, generalized seizures, cardiac failure and death. Autopsy has revealed lesions in the kidneys and the liver.

Comparing the approximate human lethal dose of 0,5-1 g/kg bw to reported LD_{50} values of diethylene glycol in different animal species, man appears to be about 10 times more sensitive than the animal species commonly used in toxicity studies. In both experimental animals and man, the kidneys, the liver and the nervous system appear to be affected. Structural and, in some species, functional damage to the heart muscle is also described.

Diethylene glycol has not been evaluated by the International Agency for Research on Cancer. No human studies on long-term exposure, reproduction effects and immunological effects are available.

Animal data

The available skin irritation studies with rats, rabbits and guinea pigs show that diethylene glycol has no or only slight skin irritating properties. In different studies with rabbits, rats, cats and dogs, undiluted diethylene glycol caused no or only slight eye irritation. In a maximisation study with guinea pigs, diethylene glycol caused no sensitisation.

The 4-hour inhalation LC_{50} of diethylene glycol in rats is higher than 4.6 g/m^3. The oral LD_{50} values for mice and rats are in the range of 15-25 g/kg bw. For guinea pigs, oral LD_{50} values are in the range of 8-14 g/kg bw, and for rabbits, cats and dogs in the range of 4-11 g/kg bw. After a single-dose oral administration of diethylene glycol, the clinical signs of toxicity are similar for mice, rats, rabbits, guinea pigs, cats and dogs, and resemble those reported for humans. Symptoms like thirst, diuresis, roughened coat, refusal of food, suppression of urine, proteinuria, prostration, dyspnea and a bloated appearance were also observed.

Oral short-term exposure to diethylene glycol has resulted in tremors, lethargy, piloerection, decreased renal function, retinopathy, increased serum glutamic oxaloacetic aminotransferase, prolonged blood coagulation time, microscopical and ultrastructural changes in the myocardium and increased mor-

tality. Rabbits exposed to a 0.5-ml mixture of equal parts of diethylene glycol and propylene glycol for 100 days developed no major dermal local macroscopic or microscopic changes. No data were available on systemic effects.

In a study on the effects of diethylene glycol (0-4% in food for 14 weeks) on the kidneys, the number of female rats with urinary 'oxalate' crystals started to increase at dose levels of 360 mg/kg bw/day. An increased number of male rats with 'oxalate' crystals was found at the highest dose level of 2,970 mg/kg bw/day only. Hydropic degeneration of the kidneys and tubular necrosis were found at dose levels of 2,970 mg/kg bw/day, and in one male rat also at 1,550 mg/kg bw/day. For the development of hydropic degeneration, the NOAEL was 300 mg/kg bw/day (0.4% diethylene glycol in food). In the same study with diethylene glycol (0-2% in food) for 225 days, the number of rats with urinary 'oxalate' crystals started to increase at dose levels of 234 mg/kg bw/day and higher, and was not increased at the dose level of 105 mg/kg bw/day. The only finding at a dietary level of 105 mg/kg bw/day was a 13-23% increase in urinary 'oxalate' excretion in the males whilst at the lowest level (51 mg/kg bw/day) no effects were observed. Hydropic degeneration or tubular necrosis was not found at any dose level (0-2% diethylene glycol in food) in the 225-day study.

The available data indicate that diethylene glycol was negative in *in vitro* tests for genotoxicity. Positive results were obtained in *in vivo* genotoxicity studies, however, at high doses of diethylene glycol. The relevance of these findings for low doses is not clear.

Oral long-term studies before 1950 revealed renal effects, bladder stones, and bladder tumours. In a number of these studies, bladder tumours were reported together with the formation of oxalate-containing bladder stones. Later studies showed that in rats exposed to diethylene glycol, bladder tumours did not develop without the presence of a foreign body such as an oxalate-containing bladder stone. It has been suggested that pre-1950 studies may have been carried out with diethylene glycol that contained significant amounts of ethylene glycol (which is metabolised to oxalate). No information is available in the literature on the occurrence of bladder stones in humans after the ingestion of diethylene glycol.

Diethylene glycol lacks carcinogenic properties after oral and dermal administration. In a 108-week carcinogenicity study with rats (purity of diethylene glycol batch 97%, ethylene glycol content was not stated), increased drinking water consumption (females 17% and males 25%), increased mortality (19/50 males compared to 13/50 in the control group), increased serum lactate dehydrogenase activity, creatinine phosphokinase activity and relative weights of the lung and decreased serum urea nitrogen (males) were observed at a dose level of

2.6 g/kg bw/day. In the urine, no changes could be detected but it was not mentioned if oxalate was measured. No bladder stones were reported. Hyperplasia of the urinary tract epithelium was not observed.

Several animal reproduction toxicity studies indicate that diethylene glycol does not adversely affect fertility up to dose levels of 3.1 g/kg bw/day in mice. At a dose of 6.1 g/kg bw/day, reduced fertility was observed in the presence of maternal toxicity. With regard to developmental toxicity, a significant decrease of mean foetal body weight in mice was seen at 10.0 g/kg bw/day in the presence of maternal toxicity. At 5,0 g/kg bw/day significant maternal toxicity was present without developmental effects. In addition, at a dose level of 6.1 g/kg bw/day in a two generation study with mice, craniofacial malformations, including exencephaly and cleft palate, and related mortality were observed in the presence of maternal toxicity. In rats, minor developmental effects (delay of development, reduced litter weight and increased skeletal variation) were seen at the oral dose level of 4.47 g/kg bw/day in the presence of maternal toxicity. Foetal malformations were not observed at dose levels up to 8,94 g/kg bw/day. From these studies, the committee concluded that the reproduction toxicological properties of diethylene glycol, if at all present, will probably be expressed only at high doses that cause maternal toxicity as well. The committee does not consider this as an indication of genotoxicity.

Chapter 8

Existing guidelines, standards and evaluations

8.1 General population

No health-based limit values for the general population were retrieved for diethylene glycol.

8.2 Working population

Currently, there is no occupational exposure limit for diethylene glycol in the Netherlands and there is no limit for diethylene glycol exposure set by the European Commission. A number of member countries of the European Union (EU) have set limit values for diethylene glycol exposure (see Table 8.1).

The Health and Safety Executive (HSE) has established an occupational exposure limit of 101 mg/m^3 for the United Kingdom.[105] This limit is based on oxaluria and kidney damage, which are considered the critical effects of diethylene glycol. It is stated that although the low volatility of diethylene glycol precludes serious risk due to vapour inhalation at room temperature, aerosols may be generated and vapour may occur if the substance is heated. An occupational exposure standard was therefore set at 101 mg/m^3 (8-hour TWA) for vapour and particulate material. A short-term limit was considered unnecessary, and the three-times rule should apply as a guide to good work practice.

Table 8.1 Existing occupational exposure limits for diethylene glycol.

Country - organisation	occupational exposure limit (mg/m³)	time-weighted average	type of limit
The Netherlands - Ministry of Social Affairs and Employment[111]	-		
United Kingdom - HSE[112]	101 (vapour and particulate material)	8 hour	WEL
Denmark[113]	11	8 hour	OEL
Germany - AGS[114]	44 176	8 hour 15 min[a]	
- DFG MAK Committee[106]	44 176	8 hour 15 min[a]	MAK STEL
Sweden[107]	45 90	8 hour[b] 15 min	OEL STEL
European Union - SCOEL[115]	-		
USA - ACGIH[108]	-		
- AIHA[110]	10 (particulate material)	8 hour	WEEL
- OSHA[109]	-		
- NIOSH[109]	-		

[a] maximum number per shift: 4, with a minimum peak interval of 1 hour
[b] skin notation attached to diethylene glycol

In Germany, the 8-hour time-weighted average (TWA) limit value is 44 mg/m³.[106] The short-time limit value (15 min average; maximum 4 times/shift; minimum interval 1 hour) is 176 mg/m³. According to the Deutsche Forschungsgemeinschaft (DFG)[67], diethylene glycol can cause kidney damage and, at high doses, the formation of bladder stones and bladder tumours. No long term toxicity data on human inhalation were noted. The oral data were used to establish the maximal accepted concentration (MAC) value. Reported NOAEL's varied largely, from 50 to 2,350 mg/kg bw. It was suggested that differences in biotransformation, *i.e.*, the formation of ethylene glycol and oxalic acid, might be responsible for this. The DFG concluded that the lowest NOAEL reported was 50 mg/kg in rats exposed for 225 days to diethylene glycol in their diet.[1] It was stated that human case reports suggested an approximately 10-fold higher sensitivity of man compared to experimental animals towards diethylene glycol toxicity. Using a margin of safety of 10, the MAC value was established at 44 mg/m³. Furthermore, the DFG concluded that diethylene glycol should be regarded as a pregnancy group C substance, *i.e.*, it is not expected to cause developmental effects

after exposure to diethylene glycol at the concentration of the occupational limit value.

In Sweden, the 8-hour TWA value is 45 mg/m^3 and the 15-min short-term exposure limit is 90 mg/m^3.[107] A skin notation is attached to diethylene glycol in Sweden.

The American Conference of Governmental Industrial Hygienists (ACGIH)[108] has not specified a threshold limit value (TLV) for diethylene glycol. The National Institute for Occupational Safety and Health (NIOSH) and the Occupational Safety and Health Administration (OSHA) have not set occupational exposure limits.[109] The American Industrial Hygiene Association (AIHA) has a Workplace Environmental Exposure Limit (WEEL) of 10 mg/m^3 (8-hour TWA) for diethylene glycol as an aerosol.[110]

Chapter 9

Hazard assessment

9.1 Hazard identification

9.1.1 Effects after acute exposure

In man, severe intoxications have occurred after acute oral ingestion of diethylene glycol. From accidental diethylene glycol poisonings, it has been estimated that the human acute oral lethal dose is about 1 g/kg bw. In animals, oral LD_{50} values range from 15-25 g/kg bw (mice and rats), 8-14 g/kg bw (guinea pigs), and 4-11 g/kg bw for rabbits, cats and dogs (Table 7.1). Apparently, the acute toxicity of diethylene glycol in man is about 10 times higher than the acute toxicity in experimental animals.

In both man and experimental animals, the kidneys and the liver appear to be the primary organs affected. The convoluted tubules become swollen and plugged with debris. Post-mortem examination showed hydropic degeneration with extensive tubular necrosis in the kidneys and centrilobular hydropic degeneration of the liver. In two cases in man, amorphous calcium deposits have been described in the renal tubuli at autopsy. However, oxalate crystals have never been found in human cases of diethylene glycol ingestion in which post-mortem analysis was carried out.

Originally, long-term animal studies with oral diethylene glycol indicated the formation of oxalate. Later, however, several studies showed that the major metabolite is 2-hydroxyethoxyacetic acid and that oxalate is a minor metabolite

if at all existing. This was explained in the literature by the presence of ethylene glycol – which is metabolised to oxalate – in the diethylene glycol originally used. Today, it is generally believed that the ether bond in diethylene glycol is hardly if at all cleaved and that 2-hydroxyethoxyacetic acid is the main metabolite. Metabolism of diethylene glycol can be inhibited by alcohol dehydrogenase inhibitors. In animal studies, this has resulted in lower toxicity of diethylene glycol. Therefore, it is also generally believed that a metabolite – rather than diethylene glycol itself – is responsible for the adverse effects on the kidneys and the liver.

Neurological effects in man have been observed 1-3 weeks postingestion and included (partial) facial paresis and ascending paralysis. Post-mortem analysis revealed axonal damage and severe demyelination of central and peripheral nerves.

9.1.2 *Effects on the skin and the eyes*

The available data indicate that exposure to diethylene glycol has no or only minor skin irritating effects in man and animals. For instance, no irritation was found on the human forearm after a 2-hour occlusive exposure, twice a day, to undiluted diethylene glycol[46] or after a 48-hour occlusive exposure to 20% diethylene glycol in petrolatum on the intact back skin of healthy individuals[47].

No studies were found on the eye-irritating properties of diethylene glycol in humans. According to Guillot *et al.* (1982)[64], undiluted diethylene glycol when applied to the eyes is well tolerated and has only minor irritating effects in animals, and 10% diethylene glycol has no irritating effects.

The available human data do not allow the evaluation of the sensitising properties of diethylene glycol in man. In guinea pigs, a maximisation study did not show diethylene glycol to cause sensitisation (BASF, 1991, cited in DFG, 1995[67]).

9.1.3 *Skin permeation*

Calculation with the model SkinPerm indicates that the maximal skin permeation is 0.10 mg/cm^2/hour under steady-state conditions when skin absorption equals systemic delivery. These results indicate that dermal exposure may substantially contribute to the body burden of diethylene glycol. This model also predicts a significant latency period of approximately three hours after onset of skin exposure before systemic delivery starts to occur.

9.1.4 *Carcinogenicity and reproduction toxicity*

Diethylene glycol was not carcinogenic in animals and had no mutagenic or genotoxic effects in vitro. In vivo, genotoxic effects were only reported in a few studies at high doses of diethylene glycol. Because of limited number of publications with adequate data, the genotoxic effects of diethylene glycol in vivo can not be assessed.

No human data exist concerning the effects of diethylene glycol exposure on fertility and development nor on the immune system. In animals, diethylene glycol reduced fertility and decreased foetal body weight at relatively high doses of 4.5 g/kg bw/day and more, in the presence of maternal toxicity. Overall, the committee concluded that toxicological effects of diethylene glycol on reproduction are only expressed at high dose levels that cause significant maternal toxicity.

9.1.5 *Other effects after long-term exposure*

No human studies exist on the adverse health effects of long-term exposure to diethylene glycol.

In a long-term study with Wistar rats, Gaunt *et al.* (1976) showed that the kidneys and the liver are the target organs after dietary administration of diethylene glycol, and that the main site of toxic action was the kidney.[1] Rats received a diet containing 0%, 0.4%, 2.0% and 4.0% diethylene glycol for 14 weeks (equivalent to 0; 300; 1,550; and 2,970 mg/kg bw/day in the male rats; and 0; 360; 1,810; and 3,680 mg/kg bw/day in the female rats); and in a second experiment 0%, 0.085%, 0.17%, 0.4% and 2.0% diethylene glycol for 225 days (equivalent to 0; 51; 105; 234; and 1,190 mg/kg bw in the male rats; and 0; 64; 126; 292; and 1,460 mg/kg bw/day in the female rats). The investigators reported increased urine volumes after concentration tests, increased oxalate excretion and urinary oxalate crystals, hydropic denegeration of the kidneys and tubular necrosis in the kidneys (Table 7.2). From these results, the committee concludes that the critical effects are the summed adverse effects of diethylene glycol on the kidneys. The committee notes that the increased urine volume after the concentration test may have been influenced to some degree by the osmotic-diuretic effect of diethylene glycol. Also, the male and the female rats were affected differently and there is no dose-response relationship for the effect of increased urine volumes. The committee also notes that the results on the number of animals with ‘oxalate’ crystals in the urine were not consistent between the male and the female rats, and that no dose-response relationship can be observed for this effect. In addi-

tion, the origin of the ‘oxalate’ crystals is unclear. This unclarity is enlarged by the fact that in fatal human cases of diethylene glycol poisoning oxalate crystals have not been detected in post-mortem examination of the kidneys. Therefore, the committee decided not to use the No Observed Adverse Effect Level (NOAEL) from the ‘oxalate’ crystals findings, but to use the NOAEL for hydropic degeneration of the kidneys as the starting point for the derivation of the health-based recommended occupational exposure limit (HBROEL). The NOAEL for this effect is 300 mg/kg bw/day (Table 7.3).

9.2 Recommendation of the health-based occupational exposure limit

The human and animal data show that the kidneys and the liver are the target organs. No human studies on adverse health effects of long-term exposure to diethylene glycol exist. In a BIBRA research report, Gaunt *et al.* (1976) described the adverse effects of dietary diethylene glycol on the kidneys and the liver of Wistar rats.[1]

The committee notes that human occupational exposure to diethylene glycol may not only occur to vapour but also to aerosols of diethylene glycol. Aerosols that are retained in the upper respiratory tract, will be moved upwards and swallowed, and enter the gastrointestinal tract. As both vapour and aerosol may cause systemic effects, the committee concludes that any adverse health effect after oral exposure is relevant for the assessment of inhalatory exposure and that the diethylene glycol feeding study by Gaunt *et al.* (1976)[1] is relevant for the health based risk assessment of inhalatory exposure to diethylene glycol. As this study is the only reliable long-term study with detailed description of adverse effects, the committee decides to use this study as the key study.

From the results of the experiments of Gaunt *et al.* (1976)[1], the committee uses the NOAEL for hydropic degeneration as the starting point for the derivation of the HBROEL. The NOAEL for this effect is 300 mg/kg bw/day (Table 7.3).

For the extrapolation to the HBROEL, the following aspects are taken into account: interindividual and interspecies, and the difference between the experimental conditions and the exposure pattern of the worker. For the interindividual variation, the committee applies a factor of 3. For the approximate 10-fold higher sensitivity of humans compared to experimental animals, the committee applies an interspecies factor of 10. Assuming complete bioavailability of diethylene glycol after both oral and inhalation exposure, the committee concludes that there is no assessment factor needed for the difference between experimental

conditions in the study by Gaunt *et al.* (1976)[1] and the exposure pattern of the worker.

For the transformation into the HBROEL, the committee uses 70 kg as the average body weight of a worker and 10 m^3 as the average respiratory volume per 8-hour working day. With the overall assessment factor of 30, the committee calculates an inhalatory HBROEL of 70 mg/m^3 as an 8 hour time-weighted average concentration. This value of 70 mg/m^3 applies to the sum of the concentrations of diethylene glycol existing as a vapour and as an aerosol.

According to the committee, exposure to an aerosol can have effects that are comparable to the effects of exposure to inhalable and respirable dust. Therefore, it is the committee's opinion that health-based occupational exposure limits for inhalable and respirable dust must be applied to aerosols of diethylene glycol.*

Diethylene glycol does not have carcinogenic properties. Reproduction toxicity was only observed at high dose levels in the presence of maternal toxicity.

Skin uptake of diethylene glycol has been demonstrated in one rat study[26] but the committee considers these data insufficient to assess whether a skin notation is warranted. Skin permeation data obtained with SkinPerm indicate that dermal exposure may substantially contribute to the body burden of diethylene glycol. As a consequence of its significant systemic toxicity, the committee recommends a skin notation for diethylene glycol.

9.3 Groups at extra risk

No groups at extra risk were identified.

9.4 Health based recommended occupational exposure limit

The Dutch Expert Committee on Occupational Standards recommends a health-based occupational exposure limit for diethylene glycol of 70 mg/m^3 as an 8-hour time-weighted average concentration, applying to the sum of the concentrations of diethylene glycol existing as a vapour and as an aerosol.

The committee also recommends to apply health-based occupational exposure limits for inhalable and respirable dust to aerosols of diethylene glycol. In addition, the committee recommends a skin notation for diethylene glycol.

* In the Netherlands, MAC values for inhalable and respirable dust existed until January 2007. At the moment, the Dutch Expert Committee on Occupational Standards is re-evaluating the scientific literature in order to recommend health-based occupational exposure limits for inhalable and respirable dust.

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A	Request for advice
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Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, a 'exposure versus tumour incidence range' and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.
The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.

Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the Committee is given in annex B.

Annex **B**

The committee

- G.J. Mulder, *chairman*
  emeritus professor of toxicology; Leiden University, Leiden
- R.B. Beems
  toxicologic pathologist; National Institute for Public Health and the Environment, Bilthoven
- P.J. Boogaard
  toxicologist; Shell International BV, The Hague
- J.J.A.M. Brokamp, *advisor*
  Social and Economic Council, The Hague
- D.J.J. Heederik
  professor of risk assessment in occupational epidemiology; Institute for Risk Assessment Sciences, Utrecht University, Utrecht
- L.A.L.M. Kiemeney
  professor of cancer epidemiology, University Medical Centre St Radboud, Nijmegen
- H. van Loveren
  professor of immunotoxocology, Maastricht University, Maastricht, and National Institute for Public Health and the Environment, Bilthoven
- T.M. Pal
  occupational physician; Netherlands Center for Occupational Diseases, Amsterdam

- A.H. Piersma
  professor of reproductive toxicology; National Institute for Public Health and the Environment, Bilthoven
- H.P.J. te Riele
  professor of molecular biology, VU University Amsterdam, Amsterdam
- I.M.C.M. Rietjens
  professor of toxicology; Wageningen University and Research Centre, Wageningen
- H. Roelfzema, *advisor*
  Ministry of Health, Welfare and Sport, The Hague
- T. Smid
  occupational hygienist - epidemiologist; KLM Health Services, Schiphol, and professor of working conditions, VU University Amsterdam, Amsterdam
- G.M.H. Swaen
  epidemiologist; Dow Benelux N.V., Terneuzen
- R.A. Woutersen
  toxicologic pathologist; TNO Quality of Life, Zeist
- P.B. Wulp
  occupational physician; Labour Inspectorate, Groningen
- E.J.M. Pennings, *scientific secretary*
  Health Council of the Netherlands, The Hague

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the establishment meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

Comments on the public review draft

A draft of the present report was released in 2006 for public review. The following organisations and persons have commented on the draft report:

- E. González-Fernández, Ministerio de Trabajo y Asuntos Sociales, Spain
- T. Scheffers, Maastricht, The Netherlands
- R.D. Zumwalde, National Institute for Occupational Safety and Health, USA

Annex **D**

Human data

Table D1 Human *in vitro* studies with diethylene glycol.

Human cell type	Procedure	Concentration tested	Effects	Reference
KB cells	-	0.1; 0.2; 0.3 M	Inhibition of the growth of the cells with increasing concentrations (dose-dependency). The inhibitory dose (ID) (concentration of a compound in growth medium that caused a 50% reduction in cell number after 72 hours of incubation) of diethylene glycol was 0.18 M.	Mochida and Gomyoda 1987[116]

Table D2 Human studies on skin and eye irritation, and skin sensitisation properties of diethylene glycol.

Humans involved/No. of humans	Procedure	Concentration /Dose tested	Results	Reference
Skin irritation and sensitization				
50 volunteers	Patch test (48 h occlusive patch test)	not reported	20% was the highest concentration of diethylene glycol (in petrolatum) found to be non-irritant after 48 h covered contact	Meneghini *et al.* 1971[47]
480 eczematous dermatitis patients	Patch test (48 h covered)	a 15% glycol mixture that contained unspecified proportions of diethylene glycol, ethylene glycol and propylene glycol in petrolatum	No skin and allergic reactions were reported	Meneghini *et al.* 1971[47]
One human (man)	Patch test (24 h covered)	5% aqueous solution of diethylene glycol	Allergic dermatitis 2-4 weeks after he had started smoking a brand of cigarettes containing diethylene glycol; the man exhibited local reactions in the patch test.	Newman 1938 cited in BIBRA 1993[48]

Humans / n = not reported	Draize test; 3 days	122 mg diethylene glycol	Mild reaction to human skin; no classification required	Drill 1976 cited in IUCLID dataset 2000[2]
Humans / n = 7 men, 5 women	Two times a day 2 h exposure to the forearm	100% diethylene glycol	Not irritating	Loeser *et al.* 1954[46]

Table D3 Human accidents with regard to acute and short-term exposure to diethylene glycol.

Humans involved/No. of humans	Procedure	Concentration/dose	Effects	Reference
Oral				
One human	Ingestion of brake fluid containing diethylene glycol	Four ounces of brake fluid containing 20% diethylene glycol	The first toxic symptoms were vomiting, diarrhoea and increased urine production. Headache, drowsiness and weakness. Partial facial paralysis, hypotension, apnoea and coma preceded death, 16 days after the dose. Liver and kidney damage and fluid in the brain and lung were the principal post-mortem findings.	Wilkinson 1967[61]
Seven children	Accidentally poisoned with diethylene glycol (a pharmaceutical manufacturing error)	unknown	After a prodromal febrile illness the children presented at hospital with vomiting, impaired consciousness, dehydration, oliguria dimishing to anuria, hepatomegaly, centrilobular degeneration, metabolic acidosis, a raised blood-urea, transaminase level and enlarged kidneys with tubular necrosis. Despite supportive treatment and, in two cases, dialysis and mechanical ventilation, all seven children died within nine days of admission. Oxidation of diethylene glycol is the more probable route of elimination. Such a pathway would be expected to result in a metabolic acidosis which is a major and consistent feature in diethylene glycol poisoning.	Bowie and McKenzie 1972[60]
57-year-old man	Swallowing an 8-ounces can of Sterno (liquid form composed of 100% diethylene glycol) in a suicide attempt	100% diethylene glycol	Two days after ingestion, he developed confusion and acute renal failure requiring haemodialysis, followed on day 8 by a delayed but rapidly evolving ascending paralysis. Pathologic examination showed severe demyelination, with lesser axonal damage, of virtually all cranial and peripheral nerves sampled and sparing of central myelin. diethylene glycol in the Sterno was considered responsible for this intoxication.	Rollins *et al.* 2002[42]
15-year-old girl	Suicidal ingestion of a brake fluid containing diethylene glycol and triethylene glycol	200 ml brake fluid containing 55% triethylene glycol and 10% diethylene glycol	Metabolic acidosis with coma; diethylene glycol is oxidized by alcohol dehydrogenase (ADH) to more toxic products, thus blockade of ADH might be beneficial. Triethylene glycol, also metabolised by ADH, is less toxic. Given 4-methylpyrazole (ADH inhibitor), the acidosis resolved and the patient recovered. 4-Methylpyrazole was suggested to be useful in diethylene glycol poisoning.	Borron *et al.* 1997[117]

Three healthy young men (average age 26 years, range 21-35) in a south Pacific island	Intentional ingestion of diethylene glycol as an ethanol substitute; each of the men consumed 2-3 cups of a 100% diethylene glycol fuel.	unknown	Two days after ingestion the men experienced epigastric pain and vomiting. Six days after the ingestion they were admitted to the hospital suffering from acute renal failure that was consistent with diethylene glycol poisoning. Their delayed presentation for medical intervention six days post-ingestion was a major contributing factor to their fatal outcomes. Symptoms generally become manifest within 24 hours of ingestions. The delayed onset of symptoms may have been secondary to the concurrent use of ethanol. Hypertension and hepatotoxicity were noteworthy complications.	Doyle *et al.* 1998[118]
-	Wine producers in Austria and Germany had illegally added diethylene glycol to wine to improve the taste	The concentration of diethylene glycol ranged mostly from 1 to 10 g/l wine, with the highest concentration being 48 g/l	Twenty one cases of renal impairment were reported in the Netherlands in 1985 attributed to wine containing diethylene glycol.	Hanif *et al.* 1995[38]; Altman et al 1986 and Lau and Weber 1987 cited in Nordic steering group for assessment of health effects of chemicals, 1998[17]
A 54-year-old man	-	unknown	The man was hospitalised for progressive uraemia with oliguria and treated with haemodialysis. Renal biopsy showed acute tubular necrosis. The renal function recovered after three weeks. Strong circumstantial evidence is delivered to support the assumption that acute renal insufficiency was caused by drinking wine contaminated with diethylene glycol.	Van Leusen and Uges 1987[119]
Seven patients	Poisoning with diethylene glycol-contaminated propolis	unknown	Liver and kidney damages, pancreatic, central and peripheral nervous system lesions as well as glomerular arteriolar hyalinosis.	Drut *et al.* 1994[41]
65-year-old alcoholic man	Intoxication with diethylene glycol	150 ml of pure diethylene glycol	The man became comatose 16 hours after ingestion, necessitating artificial respiration. The patient also developed severe metabolic acidosis with high oxalate level in the urine. After plasma alkalinisation and peritoneal dialysis the patient survived the poisoning without consequences, according to a follow-up investigation a year later.	Auzepy *et al.* 1973 cited in Berufsgenossenschaft der chemischen Industrie 1990[69]
Dermal				
Five people in a burn unit of a Spanish hospital who had burns involving from 7-62% of their body surface	A drug containing diethylene glycol and diethylene glycol stearate as a solvent was applied to their skin for up to 24 days	diethylene glycol and diethylene glycol stearate were present as a solvent for the drug 'in a proportion of 6.2 to 7.1 g/kg of substance'	In all patients, the urine volume was greatly reduced between the third and sixth day of treatment; there was further evidence of kidney failure, metabolic disturbances (acidosis) and in one person signs of liver damage. Severe central nervous system abnormalities and coma preceded death. Post-mortem examination of one patient revealed extensive kidney and liver damage. Calcium oxalate crystal were absent from the kidney.	Cantarell *et al.* 1987[59]

Table D4 Large scale accidents by ingestion of diethylene glycol as a major solvent in pharmaceuticals.

Accident description	Exposure duration	Concentration/dose	Effects	Reference
United States – 1937: diethylene glycol was used as the solvent (72%) in the formulation of elixir of sulfanilamide. Overall, 353 patients had received the drug, mainly for gonorrhea, tonsillitis, otitis and soft tissue infections	The period of exposure was probably in the order of few days	The fatal cumulative dose was about 10 teaspoonfuls in children and 20 teaspoonfuls in adults. The mean fatal dose of the elixir was 53 ml for children and 99 ml for adults. The total dose of diethylene glycol in the adults that died ranged from 14-170 ml (15.6-190 g), the average fatal dose being in the region of 1.3 g/kg bw, although some survivors tolerated much higher levels, whilst in children, the lowest fatal dose was about 4 g	One hundred and five patients died, 34 children and 71 adults. Average survival after first dose was 9 days with a range of 2 to 22 days. The earliest clinical symptoms were nausea and vomiting. Subsequently, patients developed manifestations of acute renal failure such as flank pain, polyuria, anuria, coma and occasionally seizures. After an initial increase, urine production decreased and finally stopped. The initial gastrointestinal symptoms may have limited drug absorption preventing additional deaths. Serum chemistries and urinalysis were consistent with acute renal failure. Post-mortem examination was most remarkable for “hydropic tubular nephrosis” (vacuolar nephropathy) and centrilobular hepatic degeneration. Calcium oxalate deposition in the kidneys was not noted. Subsequent animal studies clearly demonstrated that diethylene glycol, and not sulfanilamide was responsible for the toxicity.	Wax 1995[54] and Wax 1996[55]
Capetown, South Africa – 1969: seven young children ranging in age from 6 to 31 months. All the children has developed a simple febrile illness for which they were had been treated as outpatients and given one of two sedative mixtures. Both of the two sedative mixtures were supposed to have propylene glycol as their diluent. Further investigation revealed that diethylene glycol had been substituted for propylene glycol.		unknown	Fatal renal failure. Shortly after receiving the medications, the children developed vomiting, diarrhea and dehydration and were hospitalised. Anuria, acidotic breathing, hepatomegaly and unresponsiveness ensued. The patients were treated with fluid hydration and correction of the acidosis. Two of the children underwent peritoneal dialysis. Average survival time from hospital admission was four days. Post-mortem examination showed extensive proximal convoluted tubular necrosis in the kidney and centrilobular hydropic degeneration of the liver. One of the patients had evidence of renal calcium deposition.	Wax 1996[55]

India – 1986: A total of 14 patients who were administered in hospital a glycerol (9%), polyglycol (51%), diethylene glycol (18.5%), water (21%) mixture for control of intracranial pressure.	unknown	Mortality. Vomiting, diarrhoea, gastrointestinal bleeding, abdominal pain, rigidity and distension set in within four to five days after administration of the mixture. Over a further two to three days oliguria, anuria, acidosis and instability of blood pressure followed. At necropsy acute, extensive cortical necrosis was seen in the kidneys. The liver showed centrilobular necrosis. Extensive haemorrhages were seen in the adrenal medullae. Rats and rabbits fed the toxic glycerol also showed extensive renal damage. Soon thereafter, it was reported that seven cataract patients from the Indian state of Bihar had died from acute renal failure. Glycerine contaminated with diethylene glycol had been administrated to these patients as well.	Pandya 1988[120]; Wax 1996[55]
Nigeria – 1990: At the Jos University Teaching Hospital in Plateau State Nigeria, 47 children ranging in age from six to 23 months had received chloroquine, acetaminophen, promethazine and or cough mixtures for the treatment of upper respiratory tract infections and malaria. Investigation of this epidemic revealed that diethylene glycol was found in the bottles of acetaminophen syrup. The acetaminophen was normally suspended in propylene glycol. However, in these cases, diethylene glycol had been sold as propylene glycol by traders to local chemists who subsequently formulated it with the acetaminophen	unknown	The subsequent illness was characterised by nausea, vomiting, tachycardia, hyperventilation, anuria, hepatomegaly, fever, diarrhoea and convulsions. Signs on admission were tachycardia, acidotic breathing, pallor, oedema and hepatomegaly. Laboratory findings included hyperkaliemia, acidosis, elevated creatinine level and hypoglycaemia. Management consisted of correction of dehydration and acidosis plus administration of antibiotics when indicated. No dialysis was performed. Eleven children (23%) died within 24 hour of admission, 13 (28%) died within 1-3 days, and 16 (34%) died later. The longest survivor died on day 14. Seven children who were taken from hospital against medical advice died soon after. The post-mortem examination performed on three of the patients showed acute extensive cortical necrosis of the kidney and central hydropic degeneration with balloonings fatty degeneration of the liver resembling the findings seen in the other diethylene glycol mass poisonings.	Okuonghae *et al.* 1992[56]; Wax 1996[55]

Bangladesh – 1990-1992: 339 children at the two major children's hospital in the capital, Dhaka. Ingestion of an acetaminophen elixir containing diethylene glycol and unknown elixirs used to treat fever. These unknown elixirs likely consisted of acetaminophen with diethylene glycol.	unknown	Unexplained acute renal failure. The children developed hepatomegaly, oedema and hypertension, they had a high serum creatinine concentration and a low serum bicarbonate concentration. Two hundred thirty-six of these children died. An investigation revealed that 51 of the fatalities were documented to have ingested an acetaminophen elixir containing diethylene glycol and 157 ingested unknown elixirs used to treat fever. These unknown elixirs likely consisted of acetaminophen with diethylene glycol. Twenty-five of the patients developed acute renal failure while hospitalised for other illnesses at the children's hospital. They had received an acetaminophen brand from the hospital pharmacy that was later shown to contain diethylene glycol. It is likely that other children were also poisoned with the diethylene glycol-containing elixirs but were not seen at the children's hospital.	Hanif *et al.* 1995[38]; Wax 1996[55]
Haiti – 1995-1996: 109 children (aged 3 months-13 years) in Haiti; most (85%) children were aged 5 years. This accident was associated with glycerine, which was contaminated with 24% diethylene glycol used to manufacture acetaminophen syrup.	Diethylene glycol was found in patients' bottles in a median concentration of 14.4%. The median estimated toxic dose of diethylene glycol was 1.34 ml/kg (1.49 g/kg) (range 0.22-4.42 ml/kg (0.25-4.9 g/kg)); laboratory analysis for diethylene glycol in patients biologic specimens had not been performed.	Acute renal failure: most cases were characterized by a nonspecific febrile prodromal illness. Clinical symptoms included vomiting, abdominal pain, lethargy and malaise. Within 2 weeks anuric renal failure, pancreatitis, hepatitis and neurologic dysfunction progressing to coma occurred in many patients. Of 87 patients with follow-up information who remained in Haiti for treatment, 85 (98%) died; 11 of 19 patients transported to the United States for treatment died. Histopathology of kidney tissue from four patients indicated acute tubular necrosis with regeneration consistent with a toxic exposure.	Centers for disease control and prevention 1996[49]; Scalzo 1996[50]; Woolf 1998[45]; Purdy 1998[51]; Junod 2000[52]; O'Brien *et al.* 1998[37] and Anonymous 1998[53]

India – 1998: 36 children aged 2 months to 6 years, admitted to two hospitals in Delhi between 1 April and 9 June 1998; most of the children (26/36) were from the Gurgaon district in Haryana or had visited Gurgaon town for treatment of a minor illness. Cough expectorant manufactured by a company in Gurgaon was found to contain diethylene glycol (17.5% v/v). 11 children aged 2 – 42 months admitted to another hospital in New Delhi in the same period. A paracetamol elixir, consumed by the children, was found to contain diethylene glycol (2.3 – 22.3 % w/w; median 15.4 % w/w).	Unknown	Acute renal failure developed after an episode of acute febrile illness with or without watery diarrhea or mild respiratory symptoms for which the children had been treated with unknown medicines by private medical practitioners. On admission to hospital the children were not dehydrated. The children had developed severe oliguria and anuria. Median blood urea concentration was 150 mg/dl (range 79-311 mg/dl) and median serum creatinine concentration was 5.6 mg/dl (range 2.6-10.8 mg/dl). Occasionally high blood pressure was observed. Kidney biopsy showed acute tubular necrosis. Thirty-three children were known to have died (on average patients died within 7 days (range 1-24 days)) despite being treated with peritoneal dialysis and supportive therapy. 8/11 children died after acute renal failure and worsening of encephalopathy (‘*altered sensorium*’). Renal biopsy showed acute tubular necrosis affecting the proximal convoluted tubules. Liver biopsy showed focal centrizonal necrosis with fatty changes. The three patients that survived had neurological sequelae.	Singh *et al.* 2001[121]; Hari *et al.* 2006[39]
Panama – 2006: Cluster of primarily elderly patients		Acute renal failure	Barr *et al.*, 2007[122]

Table D5 Human carcinogenicity studies of diethylene glycol.

Humans involved/No. of humans	Procedure	Concentration/dose tested	Effects	Reference
Inhalation				
A total of 17 cases of gliomas were included in the study. Each case was matched with 6 controls. Matching criteria were: race and sex matched the case; year of birth was within 3 years of the case's; date of first employment at the Texas City plant for the control was before that of the case, but year of first employment at the Texas City plant was not earlier than 3 years before the case's; the date the control was last employed was later than the case's last date of employment; the control, if dead, must not have died of a malignancy.	A Texas petrochemical plant had elevated standardized mortality ratios for neoplasms of the brain. A nested case-control study has examined possible associations between gliomas of the brain and job title, departmental employment history, chemical exposure history (amongst others, diethylene glycol), geographic location within plant, dates of employment and residence near the plant. For each case and control, plant personnel completed coding sheets containing demographic data, data of each new job title or department code, job code, department code, date of each layoff, date of final termination (if no longer employed at the plant), and vital status (when known). For each job, department, major department group, or chemical exposure common to at least 4 cases, an odds ratio was calculated and tested for possible significance.	Not specified	A Texas petrochemical plant had elevated standardized mortality ratios for neoplasms of the brain. The greatest apparent risks were associated with exposure to carbon dioxide, diethyl sulfate, diethylene glycol, ethanol, ethylene, isopropanol, methane, tetraethylene glycol and vinyl acetate. However, the greatest apparent risks were also associated with first employment in the 1940s or early 1950s and the highest apparent risk was associated with residence in the area around the plant. A total of 4 cases worked in departments where diethylene glycol or tetraethylene glycol was present. Even if the association was causative, the fraction of cases attributable would be insufficient to explain the observed excess; the association may be the result of multiple significance testing. For all chemicals, there were wide confidence intervals around all of the odds ratios given; therefore, differences between odds should be interpreted cautiously. No significant differences between cases and controls were apparent in duration of exposure to any of these chemicals.	Leffingwell *et al.* 1983[63]
90 workers (56 men and 34 women (age 20-49 years)) who produced aromatic hydrocarbons from crude oil	Exposure to diethylene glycol was for 1 to 9 years (retrospective epidemiological study)	Not specified	The workers did not experience any differences in the incidence of tumours of the skin, nervous system or internal organs. It is not known how long after the exposure the workers were studied. Remark: Because of the inadequate methodology of the investigation, few conclusions can be drawn from this study.	Telegina *et al.* 1971 cited in Nordic steering group for assessment of health effects of chemicals 1998[17] and in Berufsgenossenschaft der chemischen Industrie 1990[69]

Annex **E**

Animal data

Table E1 Animal studies with regard to skin and eye irritation and skin sensitisation properties of diethylene glycol.

Species/Strain/No. per Sex per Group	Procedure	Concentration / dose tested	Results	**Reference**
Skin irritation				
Rats / n=5/dose	Covered contact with the skin for 4 hr/d for 4 days, for 1 hr/d for 6 days and for 2 hr/d for 6 days	100% diethylene glycol	No irritant effects were reported	Loeser *et al.* 1954[46]
Rats	Covered contact with the skin for 2 h and for 2*2 hr/d for 2 days	25 ml/kg 100% diethylene glycol	No irritant effects were reported	Loeser *et al.* 1954[46]
Five female rabbits	Daily treatment for 100 days with a mixture of equal parts of diethylene glycol and propylene glycol	a mixture of equal parts of diethylene glycol and propylene glycol (0.5 ml)	There were no macroscopic changes. Microscopic examination after 20-30 days showed a slightly thickened stratum granulosum and signs of proliferation in the stratum basale. Superficial portions of the dermis showed some infiltration with cells of the lymphatic series and histiocytes. The collagen fibres were slightly fragmented and scattered. The findings remained unaltered in later stages. Thus, the glycols seemed not to produce major changes in rabbit skin, not even after prolonged periods of application.	Rantuccio *et al.* 1979[65]
Rabbits	Occlusive contact with the skin	10 and 100% diethylene glycol	No irritant effects were reported	Guillot *et al.* 1982[64]
Rabbits / n=3	Application of diethylene glycol to the uncovered skin of rabbits daily for 6 weeks (2 ml)	10 and 100% diethylene glycol	100% diethylene glycol produced only a very slight irritation. No significant adverse reaction was observed macroscopically and histologically; a 10% aqueous solution was totally without irritant action in three rabbits.	Guillot *et al.* 1982[64]
Rabbits	Skin irritation study (Draize test); application of 0.5 g	diethylene glycol	No irritant effects were reported.	Deichmann 1969 cited in Nordic steering group for assessment of health effects of chemicals 1998[17]

Guinea pigs	Covered contact with the skin for 2 hr, for 2*2 hr/d for 4 days and for 2*2 hr/d for 14 days	25 ml/kg 100% diethylene glycol	No irritant effects were reported.	Loeser *et al.* 1954[46]
Skin sensitisation				
Guinea pigs	Maximisation test according to Directive 84/449/EEC, B.6	diethylene glycol	No sensitisation was reported	BASF 1991 cited in Deutsche Forschungsgemeinschaft (DFG) 1995[67]
Eye irritation				
Rabbits	Draize test	50 mg 100% diethylene glycol	Slightly irritating; no classification required	Anonymous 1931 cited in IUCLID dataset 2000[2]
Rabbits	Eye irritation study	100% diethylene glycol	No irritant effects were reported	Loeser *et al.* 1954[46]
Rabbits	Eye irritation study	100% diethylene glycol (0.5 ml)	No irritant effects were reported	Carpenter and Smyth 1946[66]
Rabbits	Eye irritation study	10 and 100% diethylene glycol	100% diethylene glycol was slightly irritating	Guillot *et al.* 1982[64]
Rats, cats and dogs	Eye irritation study	100% diethylene glycol	No irritant effects were reported	Loeser *et al.* 1954[46]

Table E2 Acute animal toxicity studies of diethylene glycol.

Species/Strain/No. per Sex per Group	Exposure duration	Concentration / dose tested	NOAEL[a]	LOAEL[b]	(Critical) effects	Reference
Inhalation						
Mice	2 hr	130 mg/m^3	-	130 mg/m^3	Remark: Evaluation of the results is not possible because the study is described in Russian. However, in two review articles the following effects are mentioned: mortality, excited behaviour, general anaesthetic response and cyanosis (BIBRA, 1993.[48]) and changes in kidneys and liver (Nordic steering group for assessment of health effects of chemicals, 1998.[17])	Sanina and Kocketkova, 1966 cited in BIBRA 1993[48] and in Nordic steering group for assessment of health effects of chemicals 1998[17]
Rats	4 hr	4.4-4.6 g/m^3 (maximum attainable concentrations) (the MMAD of particles ranged from 2.6-3.1 µm and greater than 96% of the particles were below 10 µm)	4.4-4.6 g/m^3	-	Decreased activity during exposure with rapid recovery on removal, a transient body weight loss with recovery in 3-5 days, and nasal discharge or lacrimation suggestive of minor irritation which persisted for several days. Post mortem examinations were unremarkable. LC_{50} was therefore higher than 4.6 g/m^3	Cascieri *et al.* 1991[68]
Mice (n=6)	8 hr	Saturated vapour (not further defined)	not specified	not specified	All mice survived	Union Carbide, 1958 cited in BIBRA 1993[48]
Oral						
Mice	once	15-35 ml/kg	-	> 20 g/kg bw	LD_{50} > 20 g/kg bw	Laug *et al.* 1939 and Meyer and Stürmer 1952 cited in BIBRA 1993[48]
Mice	once	not reported	-	13-27 g/kg bw	Mortality (LD_{50})	Criteria group for occupational standards 1993[18]
Mice	single dose	26.5 g/kg	-	26.5 g/kg bw	Mortality (LD_{50})	Williams *et al.* 1990[20]
Rats	once	15-35 ml/kg	-	16.6 g/kg bw	Mortality (LD_{50})	Laug *et al.* 1939 cited by Lenk et al 1989[30]
Rats	once	not reported	-	20.8 g/kg bw	Mortality (LD_{50})	Smyth *et al.* 1941 cited by Lenk et al 1989[30]
Rats	once	not reported	-	15.6 g/kg bw	Mortality (LD_{50})	Plugin 1968 (Russian) cited by Lenk *et al.* 1989[30]

Rats	once	not reported	-	16-21 g/kg bw	Mortality (LD_{50})	Criteria group for occupational standards 1993[18]
Rats / n=10/dose	once by gavage	1, 5, 10, 12.5, 15 and 17.5 ml/kg bw (1.12-19.5 g/kg bw)	-	17.5 ml/kg bw (19.5 g/kg bw)	Mortality (LD_{50})	Lenk *et al.* 1989[30]
Rats (male)	once by gavage	15 ml/kg bw (16.7 g/kg bw)	-	15 ml/kg bw (16.7 g/kg bw)	Mortality (LD_{50})	Fitzhuhg and Nelson 1946 cited in Hebert *et al.* 1978[123]
Rats (SD) / n=12/dose	once by gavage	0 (2 animals), 6, 12 and 24 ml/kg bw (0, 6.7, 13.4 and 26.8 g/kg bw)	-	6 ml/kg bw (6.7 g/kg bw)	Ataxia, lethargy, dyspnoea. The animals exposed to 6 and 12 ml/kg bw (6.7 and 13.4 g/kg bw) gained normal activity within 6 to 8 h post-dosing. 8/24 animals exposed to 24 ml/kg bw (26.8 g/kg bw) died prior to the 48 h sacrifice time; 4 of these were active and alert at 36 h post-dosing. All of the animals exposed to 24 ml/kg bw (26.8 g/kg bw) were alert and active for at least 6 h post-dosing. All exposed animals showed hydropic degeneration with protein disposition and variation in cell size in the kidneys without oxalate deposition.	Winek *et al.* 1978[21]
Rats / n=10/dose	once by gavage	1, 5, 10, 12.5, 15 and 17.5 ml/kg bw (1.12-19.5 g/kg bw)	-	1-17.5 ml/kg bw (1.12-19.5 g/kg bw) LD_{50} (19.6 g/kg bw)	Initially, narcotic phase, diuretic phase (resulting from the hygroscopic properties of diethylene glycol), thirst, drop of urinary and blood pH; depending on the dose, either recovery to normal or hydrotropic degeneration of the renal tubules and anuria, accumulation of blood urea and uric acid, renal failure due to obstructed urine flow (profound swelling of the epithelium of the convulated tubules); finally, death after 2-7 days from non-compensated metabolic acidosis and renal failure. At 17.5 ml/kg bw (19.5 g/kg bw) diethylene glycol, death also occurred in the acute phase of intoxication, *i.e.*, within 24 h, possibly by a direct damaging effect. Narcotic and diuretic effects increased with increasing dose.	Lenk *et al.* 1989[30]

Rats	once by gavage	1, 5, 10, 15 and 17.5 ml/kg bw (1.12-19.6 g/kg bw)	-	1-17.5 ml/kg bw (1.12-19.6 g/kg bw) LD_{50} (19.6 g/kg bw)	linear increase of 24-h urine volume with dose; oral doses of 19.6 g/kg further increased the urine volume in 3 of 10 animals, indicating a limitation of the renal excretory capacity. Doses of 16.7 g/kg and 19.5 ml/kg diethylene glycol produced a 4-fold increase in the 24-h urine volume as compared to controls. Polyuria followed by oliguria/anuria after 24-48 h. Oral doses of 19.5 g/kg diethylene glycol produced oliguria/anuria, uraemia, tremors, spasms, unpleasant body odour, cold limbs, ruffled coat, turbid and pale red lens in five of ten rats; they died 1-2 days later in uremic coma. Diethylene glycol produced a dose-dependent metabolic acidosis with high lactate levels.	Heilmair *et al.* 1993[22]
Rats (Wistar; males and females)	once	2% LD_{50} and 5% LD_{50} (specific LD_{50} value not reported)	-	2% LD_{50}	The levels of cytochrome P-450 were essentially unchanged both in male and female rats. However, a series of drug-metabolising mixed function oxidases were found to be decreased dose-dependently in female rats and increased dose-dependently in male rats, whereas the activities of nitroanisole-O-dealkylation, aniline-hydroxylation, and biphenyl-4-hydroxylation were increased dose-dependently in the livers of male and female rats; a dose-dependent decrease of 3,4-benzo(a)-pyrene-hydroxylation and acetanilide-4-hydroxylation could be observed both in male and female rats.	Achatz and Janik 1988[124]
Rats (SD) / n=6 females/dose	once by gavage	0.2, 0.7, 2.0 and 8.0 g/kg bw	0.2 g/kg bw	0.7 g/kg bw	Study focussed on renal impairment. 0.7 g/kg bw: LDH activity was significantly enhanced one day after treatment 2.0 g/kg bw: additional rise in urinary GAL activity two days after treatment; significant rise of urinary volume and a decrease in creatinine concentration and pH on the first day 8.0 g/kg bw: in addition to the changes mentioned above, after one day, leucine aminopeptidase activity was significantly elevated and the specific gravity decreased. However, in all animals the wet weight of the kidneys remained normal as compared to controls. The results thus show transient dose-dependent changes in several renal parameters, indicating a slight-to-moderate and reversible renal impairment.	Freundt and Weis 1989[78]

Rats (Wistar; males) / n=26	once by gavage	15 ml/kg (16.7 g/kg bw)	-	15 ml/kg bw (16.7 g/kg bw)	Observations in 57% of the animals: severe metabolic acidosis, tubular necrosis, vacuolar degeneration resulting in the formation of dilated tubes and deposit of calcium 'oxalate' crystals; spontaneous mortality: 66%; no particular effect on the myocardial fibre, only a few clusters of 'oxalate' crystals were noted within the myocardium and capillary congestion, more evident in the sub-epicardium.	Hebert *et al.* 1978[123]
Rabbits	once	not reported	-	4.40 ml/kg bw (4.9 g/kg bw)	Mortality (LD_{50})	Laug *et al.* 1939 cited in Berufsgenossenschaft der chemischen Industrie 1990[69]
Guinea pigs	once	not reported	-	9-14 g/kg bw	Mortality (LD_{50})	Criteria group for occupational standards 1993[18]
Guinea pigs	once	5-20 ml/kg	-	7.76 ml/kg bw (8.7 g/kg bw)	Mortality (LD_{50})	Laug *et al.* 1939 cited in Berufsgenossenschaft der chemischen Industrie 1990[69]
Guinea pigs, rabbits and dogs	once	5-20 ml/kg	-	4-17 g/kg bw	Mortality (LD_{50})	Laug *et al.* 1939 and Smyth *et al.* 1941 cited in BIBRA 1993[48]
Hamsters	once	not reported	-	>7.5 g/kg bw	Mortality (LD_{50})	Yoshida *et al.* 1986[88] (only tabulated data in English)
Cats	once	not reported	-	3.7-5.3 g/kg bw	Mortality (LD_{50})	Laug *et al.* 1939 cited in BIBRA 1993[48]
Cats	once	not reported	-	9 g/kg bw	Mortality (LD_{50})	IUCLID dataset, 2000[2]
Dermal						
Rats (s.c.)	single dose	not reported	-	16.8 ml/kg bw (18.7 g/kg bw)	Mortality (LD_{50})	Union Carbide Corporation, unpublished data cited in Cavender and Sowinski, 1994[125]
Rabbits	once (24-h covered contact)	not reported	-	13.1 g/kg bw	Mortality (LD_{50})	Union Carbide, 1958 cited in BIBRA 1993[48]
Rabbits	once (occlusive application)	not reported	-	11.9 ml/kg bw (13.3 g/kg bw)	Mortality (LD_{50})	Rowe and Wolf, 1982 cited in Berufsgenossenschaft der chemischen Industrie 1990[69]

Rabbits	once	not reported	-	12500 – 13300 mg/kg bw	Mortality (LD_{50})	Nordic steering group for assessment of health effects of chemicals 1998[17]
Dogs / n=10		9 ml/kg bw (10 g/kg bw)	-	9 ml/kg bw (10 g/kg bw)	Weakness in hind legs, drunken gait, general lassitude, loss of appetite, increased respiration, diuresis, vomiting, thirst, anuria, coma, muscular tremors, spasms, delirium and death	Geiling *et al.* 1937[103]
Other routes						
Rats (i.p.)	single dose	not reported	-	6.86 ml/kg bw (7.6 g/kg bw)	Mortality (LD_{50})	Union Carbide Corporation, unpublished data cited in Cavender and Sowinski 1994[125]
Rats (i.p.)	single dose	0.25, 0.5 and 0.75 ml/100 g bw in saline	0.25 ml/100 g bw	0.5 ml/100 g bw	Dose-dependent proteinuria; oliguric effect; increased excretion of free hydrogens ions; compensated impairment of renal tubular transport processes	Kraul *et al.* 1991[104]
Rats (female Wistar) (i.p.)	single dose	2.8, 5.6, 8.3 and 11 g/kg bw diethylene glycol (purity not stated) in saline	2.8 g/kg bw	5.6 g/kg bw	Nephrotoxic effects after 4-8 days; urinary protein, urinary volume and secretion of free hydrogen ions were enhanced. At 11 g/kg bw: convulsions and death.	Kraul *et al.* 1991[104]
Rats (i.p.)	single dose	2.5 – 7.5 g/kg bw	-	2.5 g/kg bw	Proteinuria, reduced urine flow, increased excretion of hydrogen ions and inhibited tubular transport. Kidney effects reached a maximum 4-8 days after the dose	Lindl *et al.* 1986 cited in Criteria group for occupational standards 1993[18]
Mice (i.p.)	single dose	not reported	-	9.7 g/kg bw	Mortality (LD_{50}); damage to the spleen, thymus, renal glomeruli and tubules, high white cell count and pulmonary congestion	Karel *et al.* 1947 cited in Cavender and Sowinski 1994[125]
Rats (i.v.)	single dose	not reported	-	8.0 ml/kg bw (8.9 g/kg bw)	Mortality (LD_{50})	Union Carbide Corporation, unpublished data cited in Cavender and Sowinski 1994[125]

[a] NOAEL = No Observed Adverse Effect Level
[b] LOAEL = Lowest Observed Adverse Effect Level

Table E3 Short-term animal toxicity studies of diethylene glycol.

Species/Strain/No. per Sex per Group	Exposure duration	Concentration/dose tested	NOAEL[a]	LOAEL[b]	(Critical) effects	Reference
Inhalation						
Rats	5 days/week for 6 months	0.004-0.005 mg/l and 0.02-0.03 mg/l	0.004-0.005 mg/l	0.02-0.03 mg/l	Remark: no information available on the original study and the critical effects.	IUCLID dataset 2000[2]
Oral						
Mice / n=4/sex/dose	14 days	1, 2.5, 5, 7.5 and 10% diethylene glycol (purity >99%) in drinking water (equivalent to 0, 2.6, 6.5, 13, 19.5 and 26 g/kg bw/day)	6.5 g/kg bw/day	13 g/kg bw/day	at 5%: decreased body weight gain; water consumption and dehydration in females; at 7.5%: decreased body weight gain, piloerection, tremor, lethargy and mortality (3/8) in males; dehydration in females; at 10%: decreased body weight gain, piloerection, tremor, ataxia, hyperactivity, lethargy and mortality in 2 of 8 females and in 3 of 8 males;	Williams *et al.* 1990[20] and NTP 1991[100]
Mice (NMRI) / n=20/dose	4 months (14-17 weeks)	0%, 0.03%, 0.3% and 3% diethylene glycol in drinking water (0, 50, 500 and 5000 mg/kg bw/day); one week after start of the experiment, animals were immunised with tetanus toxoid, Vaccinia virus and human erythrocytes.	-	50 mg/kg bw/day	The coagulation time was significantly prolonged at 50 (27%), 500 (46%) and 5000 (72%) mg/kg bw/day. After 2.5 months of exposure, elevated serum aspartate aminotransferase (ASAT) activity was observed (middle dose group). After 3.5 months, the two higher dose groups had a statistically significant (50%) reduction in serum titre of tetanus antibodies, indicating reduced immune defence, although a clear dose-response relationship was lacking. No effects were seen on the delayed type hypersensitivity reaction towards tetanus toxoid and human erythrocytes. After 4 months exposure, all the mice were inoculated with *Streptococcus pyogenes*. Exposure to 500 mg/kg/day diethylene glycol significantly enhanced streptococcus induced mortality in mice. At 5000 mg/kg/day, streptococcus induced mortality in mice was not increased. Because of the absence of a dose-relationship, the relevance of this effect is not clear.	Huber *et al.* 1986[79]
Rats / n=2-7/sex/dose	2-8 days; 3 times daily	0.5-4 ml (1680, 7560, 10080 and 20070 mg/kg bw) diethylene glycol ('pure', not further specified) by gavage	1680 mg/kg bw	7560 mg/kg bw	Thirst; diuresis; kidney failure; rapid breathing; coma; mortality after 2-5 days; hydropic degeneration of tubules epithelium of the kidneys; necrosis of kidney cells	Geiling *et al.* 1937 and Cannon 1937 cited in Deutsche Forschungsgemeinschaft (DFG) 1995[67]

Rats	8 days	Divided daily doses of 0.5-4 ml	0.5 ml (0.56 g)	-	Doses equivalent to 2 ml/kg/day (2.2 g/kg/day) killed all the animals (total dose 14-18 ml (15.6-20 g)), with symptoms matching those described after single oral doses. Similar effects were also observed in groups of rabbits and dogs treated with diethylene glycol or the Massengill elixir	Geiling *et al.* 1937 cited by Hesser 1986[126]
Rats (n=5)	11-50 days	Daily single or divided doses of 7.5 ml/kg/day	-	7.5 ml/kg/day (8.4 g/kg/day)	All rats died; apart from a few exceptions, the liver and kidneys exhibited vacuolar degeneration.	Weatherby and Williams 1939 cited in Berufsgenossenschaft der chemischen Industrie 1990[69]
Rats / n=3-31/sex/dose	1-35 days	1120-28000 mg/kg bw by gavage	-	1120 mg/kg bw	1120-8400 mg/kg bw: kidney lesions; degenerated and necrotic tubulus cells in the kidneys; oedema in the liver 3360 mg/kg bw: trombose in kidney veins and hydropic degeneration in the liver 11200 mg/kg bw and higher: lethality (100%); hydropic degeneration in the kidneys	Harris 1949 cited in Deutsche Forschungsgemeinschaft (DFG) 1995[67]
Rats	20 days	3.1 g/kg/day	3.1 g/kg/day	-	No cumulative effects; diethylene glycol may be readily metabolised under the conditions of this study	Plugin 1968 (Russian) cited by Cavender and Sowinski 1994[125]
Rats (Wistar) / n=5/sex/dose (highest dose group: 5 additional animals were examined after exposure for 3 weeks)	28 days	0, 500, 2500, 10000 and 40000 mg/kg diethylene glycol (purity 99%) in the diet (38, 188, 750 and 3000 mg/kg bw/day)	10000 mg/kg diet (750 mg/kg bw/day)	40000 mg/kg diet (3000 mg/kg bw/day)	A significant concentration and overall amount of oxalate was found in the urine of both sexes. In males also oxalate stones were observed in the urine. On discontinuation of treatment, calcium oxalate excretion was reversible.	BASF AG 1988 cited in Berufsgenossenschaft der chemischen Industrie 1990[69]
Rats / n=6sex/dose	50 days; 5 times weekly	112, 560, 1120 and 2240 mg/kg bw by gavage	2240 mg/kg bw	-	-	Loeser *et al.* 1954[46]
Rats	11 days-3 months	1-2 g/kg bw/day and more in drinking water and by gavage	-	1-2 g/kg bw/day	Kidney and liver damage	Loeser *et al.* 1954 cited in BIBRA 1993[48]

Rats / n=6/sex/dose	3 months	1%, 2%, 5%, 10% and 20% diethylene glycol ('pure', no additional information) in drinking water	1%	2%	The rats in the groups which received the 5, 10 and 20% solutions exhibited, in the course of 3-5 days, rapid weight loss, stupor, increasing weakness and reduced reaction to external stimuli. There was also haemoglobinuria and severe diuresis, followed by exsiccosis. Splenatropy was evident on dissection. Microscopically, the liver cells were reduced in size and more densely packed and in the kidney the tubular epithelium was severely swollen with vacuolisation and sporadic necrosis.	Loeser *et al.* 1954 cited in Cavender and Sowinski 1994[125], in Deutsche Forschungsgemeinschaft (DFG) 1995[67] and in Berufsgenossenschaft der chemischen Industrie 1990[69]
Rabbits (New Zealand) / n=4/dose	3 months	0% and 4% (about 7 g/kg bw/day) diethylene glycol (purity not stated) in drinking water	-	7 g/kg bw/day	This study focused on the effect of diethylene glycol on the retina. The electro-retinogram revealed a dose-dependent prolongation of the b-wave and a reduction in the photopic amplitudes of the a- and b-waves. In agreement with the electrophysiological findings, the histological study of the retina showed birefringent crystals predominantly in the inner nuclear cell layer.	Rossa and Weber 1987[80]
Rats / n=5 males	9 weeks	0.125, 0.25, 0.5, 1, 2, 4%, 5%, 10% and 20% diethylene glycol (purified substance; no further specification) in drinking water	0.125% diethylene glycol in drinking water	0.25% diethylene glycol in drinking water	0.25%; decreased body weight gain 1%: slight myocard damage 4%: mortality; irritation in gastro-intestinal tract 5-20%: mortality within two weeks Remark: Precise test details and pathological-histological organ changes are not given.	Holck 1937 cited in Deutsche Forschungsgemeinschaft (DFG) 1995[67]
Rats (Wistar) / n=10 males	up to 60 days	8400 mg/kg bw (diethylene glycol purity > 98%)	-	8400 mg/kg bw	Mortality; kidney and liver damage (vacuolar degeneration)	Weatherby and Williams 1939 cited in Deutsche Forschungsgemeinschaft (DFG) 1995[67]

Rats / n=17-35/dose	1-174 days	Diethylene glycol (purity not stated) in drinking water: 17 rats received 0,5% (300 mg/kg bw/day) over 33-124 days, 30 rats received 1% (600 mg/kg bw/day) over 33-174 days, 25 rats received 3% (3500 mg/kg bw/day) over 15-95 days and 35 rats recieved 5% (6000 mg/kg bw/day) over 1-6 days	600 mg/kg bw	3500 mg/kg bw	At 3500 mg/kg bw/day, 14/25 rats died after 5-56 days. At 6000 mg/kg bw/day, 9/35 rats died after 1-6 days. Extensive epithelial lesions in the renal tubules with retention of urine, increased residual nitrogen and uraemia were observed in animals at the two highest dose groups. Vacuolar degeneration was found in the liver and adrenocortical cells.	Kesten *et al.* 1937 cited in Hesser 1986[126], in Deutsche Forschungsgemeinschaft (DFG) 1995[67] and in Berufsgenossenschaft der chemischen Industrie 1990[69]
Rats (SD) / n=8 females	90 days; daily dosing via drinking water	0 and 0.2 g/kg bw diethylene glycol (analytical grade)	0.2 g/kg bw	-	Study was focussed on renal impairment. No change in renal function	Freundt and Weis 1989[78]
Rats / n=10/sex/dose	100 plus additional 75 days	0.3% and 1% diethylene glycol (purity not stated) (0.59 and 1.94 g/kg bw/day) in drinking water	-	0.3% (0.59 g/kg bw/day)	Two rats in each group died during the first 100 days. None of the rats treated for an additional 75 days died	Weatherby and Williams 1939 cited in Hesser 1986[126]
Rats / n=45/sex/dose	6 months; two times a week	2500 and 5000 mg/kg bw; 2 times a week by gavage (purity not stated)	5000 mg/kg bw	-	-	Loeser *et al.* 1954[46]
Rabbits / n=1-2/sex/dose	up to 9 days	1680 and 3360 mg/kg bw by gavage	-	1680 mg/kg bw	General weakness, increased breathing, anuria, kidney failure, coma, death	Geiling *et al.* 1937 cited in Deutsche Forschungsgemeinschaft (DFG) 1995[67]
Rabbits / n=6	28 days	1-4 g/kg bw diethylene glycol (purity not stated) in drinking water	-	1 g/kg bw	One rabbit died after intake of 1 g/kg bw for 7 days (kidney damage, pulmonary oedema). 4/5 rabbits, killed after 28 days, exhibited kidney lesions (vacuolar degeneration, necrosis and calcification of the tubules) and 2 of them also exhibited liver lesions (vacuolar degeneration).	Kesten *et al.* 1939 cited in BIBRA 1993[48] and in Berufsgenossenschaft der chemischen Industrie 1990[69]

Guinea pigs / n=9 males	2-11 days (sublethal dose); once daily	1.2 g/kg bw diethylene glycol (ethylene glycol content: 0.4%)	-	1.2 g/kg bw	Study focussed on microscopy and ultrastructural changes of the myocardium: coagulative myocytolysis and loss of myofibrils was patchily distributed throughout the myocardium. The accompanying ultrastructural features included swelling, pleomorphism and hyperplasia of mitochondria with an associated distension of interfibrillary spaces and a displacement, distortion and rupture of adjacent myofibrils. Histological renal changes were similar to those reported in previous investigations	Ogbuihi *et al.* 1991[81]
Guinea pigs / n=5	2-12 days	2-5 ml/kg	-	2 ml/kg (2.2 g/kg)	Kidney lesions in all animals and liver lesions (vacuolar degeneration) in 2 of them.	Kesten *et al.* 1939 cited in Berufsgenossenschaft der chemischen Industrie 1990[69]
Hamsters / n=4/dose	3 weeks	1, 2%, 3%, 4% and 5% diethylene glycol in drinking water	-	-	No deaths occurred at 2%; deaths were reported at 3%	Yoshida *et al.* 1986[88] (only tabulated data in English)
Dogs / n=3	up to 13 days	8400 mg/kg bw by gavage	-	8400 mg/kg bw	Mortality, liver and kidney damage	Weatherby and Williams, 1939 cited in Deutsche Forschungsgemeinschaft (DFG) 1995[67]
Dogs / n=5	Daily doses of 5.25 ml/kg/day up to 18 days	5.25 ml/kg/day (5.9 g/kg/day)	-	5.25 ml/kg/day (5.9 g/kg/day)	Dogs died after cumulative doses of 21-94.5 ml/kg (23.4-105.4 g/kg) (4-18 days). Forty daily doses of 5.25 ml/kg/day (5.9 g/kg/day) were not fatal in rats, however.	Weatherby and Williams, 1939 cited in Hesser 1986[126]
Dermal						
Mice	2 months	2800 mg/kg bw/day diethylene glycol (purity not stated)	-	2800 mg/kg bw/day	Evaluation of the results is not possible because the study is described in Russian. In reviews, however, the following effects are mentioned: oedema and hyperaemia in the brain and spinal cord; localized tissue bleeding in the brain; destruction of neurons with compensatory outgrowth of glial cells; Study is of limited value because of insufficient documentation of test set-up and results.	Marchenko, 1973[127] cited in Deutsche Forschungsgemeinschaft (DFG) 1995[67] and in Berufsgenossenschaft der chemischen Industrie 1990[69]
Rabbits / n=3/dose	30 days; 1 hour/day	0.16 or less and 0.32 or more ml/kg bw/day	0.16 ml/kg bw/day (0.18 g/kg bw/day)	0.32 ml/kg bw/day (0.36 g/kg bw/day)	Mortality (3/3 animals died after 21-25 days)	Hanzlik *et al.* 1947 cited in Criteria group for occupational standards 1993[18]

Rabbits / n=5 females	Daily treatment for 100 days with a mixture of equal parts of diethylene glycol and propylene glycol (exposure area 100 cm^2; no specification of exposure time per day and whether the substance was removed after each day).	a mixture of equal parts of diethylene glycol and propylene glycol (0.5 ml)	0.5 ml of a mixture of diethylene glycol and propylene glycol (1:1)	-	There were no macroscopic changes. Microscopic examination after 20-30 days showed a slightly thickened stratum granulosum and signs of proliferation in the stratum basale. Superficial portions of the dermis showed some infiltration with cells of the lymphatic series and histiocytes. The collagen fibres were slightly fragmented and scattered. The findings remained unaltered in later stages. From the study description, no data are available on systemic effects.	Rantuccio *et al.* 1979[65]
Other routes						
Rats	3 months daily; i.p. injection	300 mg/kg	-	300 mg/kg	Brain oedema, plethora of brain tissue, petechial haemorrhages and irregular distribution of cytoplasmic RNA. Histologically, some degenerative changes and vacuolisation were seen.	HSDB 2003[40]

[a] NOAEL = No Observed Adverse Effect Level
[b] LOAEL = Lowest Observed Adverse Effect Level

Table E4 Long-term animal toxicity studies of diethylene glycol.

Species/Strain/No. per Sex per Group	Exposure duration	Concentration/dose tested	NOAEL[a]	LOAEL[b]	(Critical) effects	Reference
Inhalation						
Mice and rats	3-7 months	5 mg/m³ (0.005 mg/l) diethylene glycol (purity not stated)	-	5 mg/m³ (0.005 mg/l)	Evaluation of the results is not possible because the study is described in Russian. In reviews, however, the following effects are mentioned: oedema and hyperaemia in both brain and spinal cord; localized bleedings in the brain; destruction of neurons with compensatory outgrowth of glial cells.	Marchenko 1973[127] cited in Cavender and Sowinski 1994[125], in BIBRA 1993[48], in Anonymous[67] and in Berufsgenossenschaft der chemischen Industrie 1990[69]
Mice / n=16	2 hour/day; 6-7 months	Damp aerosol mist at 30-35 °C; 4-5 mg/m³ (ca. 0.92 ml/m³) diethylene glycol (purity not stated)	-	4-5 mg/m³	Evaluation of the results is not possible because the study is described in Russian. Different reviews mention the following effects: bronchitis, pneumonitis, liver (slight protein dystrophy) and kidney damage (dystrophy of the epithelium and round-cell infiltrations). Ten out of 16 animals developed tumours 2.5 to 11 months after the end of the experiment: lymphosarcoma in the back of the neck in 1 mouse; smooth-cell, non-keratinizing tumour of the mammary gland in 1mouse; adenocarcinomas of the mammary glands in 7 mice; solid tumour (not further defined) in 1 mouse. Reviews state that this study is of limited value because: - lack of information on the analysis of the aerosol-vapour mixtures, including diethylene glycol concentrations; - lack of information on the control animal housing at elevated temperatures; - no records of tumour incidence in 20 untreated controls.	Sanina 1968[128] cited in BIBRA 1993[48], in Cavender and Sowinski 1994[125], in Deutsche Forschungsgemeinschaft (DFG) 1995[67] and in Berufsgenossenschaft der chemischen Industrie 1990[69]

Oral						
Rats / n=15/sex/dose	99 days	0%, 0.4%, 2.0% and 4.0% in the diet (diethylene glycol batch contained less than 0.01% ethylene glycol) (equivalent to 0, 300, 1600 and 3000 mg/kg bw/day for males and 0, 400, 1800 and 3700 mg/kg bw/day for females).	see Table 7.3	see Table 7.3	A level of 4.0% caused the death of six male rats with signs of renal damage. The survivors at this dose level showed a reduced growth rate, increased water intake, increased urinary flow, signs of haemoconcentration, enlarged kidneys, and renal and hepatic damage. Dietary concentrations of 0.4 and 2.0% resulted in 'oxalate' crystaluria and mild defects of renal function as measured by concentration tests. The only finding at a dietary level of 0.17% was a 13-23% increase in urinary 'oxalate' excretion in males whilst the lowest level (0.085%) no effects were observed.	Gaunt *et al.* 1976[129,1]
Rats (n=10/sex/dose)	225 days	0%, 0.085%, 0.17%, 0.4% and 2.0% in the diet (diethylene glycol batch contained less than 0.01% ethylene glycol) (equivalent to 0, 50, 100, 230 and 1200 mg/kg bw/day for males and 0, 60, 130, 290 and 1500 mg/kg bw/day for females)				
Rats	3-7 months	300 mg/kg bw diethylene glycol (purity not stated) by gavage	-	300 mg/kg bw	Remark: Evaluation of the results is not possible because the study is described in Russian. However, in different review articles the following effects are mentioned: Oedema and hyperaemia both in the brain and in the spinal cord, localized tissue bleeding in the brain, destruction of neurons with compensatory outgrowth of glial cells. xThese different review articles also state that this study is of limited value because:	Marchenko 1973[127] cited in Deutsche Forschungsgemeinschaft (DFG) 1995[67] and in Berufsgenossenschaft der chemischen Industrie 1990[69]

					As there is insufficient documentation of the test set-up and results, no evaluation of the findings is possible.	
Rats (Fisher 344) / n=20 males	30 weeks	2.5% diethylene glycol (diethylene glycol purity 97%, ethylene glycol content was not stated) in drinking water (2600 mg/kg bw/day)	2600 mg/kg bw/day	-	No renal promoting potential was evident after initiation with N-ethyl-N-hydroxyethylnitrosamine for two weeks.	Hiasa *et al.* 1990[19]
Rats / n=5 males	up to 24 months	0.79 and 1% diethylene glycol (no information on purity) (1310 and 2560 mg/kg bw/day) in drinking water	-	1310 mg/kg bw/day	1310 mg/kg bw/day: kidneys: tubular hydropic degeneration; 'oxalate' crystals in the tubules; liver: centrilobular hydropic degeneration; 2560 mg/kg bw/day: mortality; decreased body weight	Hanzlik *et al.* 1947 cited in Deutsche Forschungsgemeinschaft (DFG) 1995[67]
Rats / n=6 males and 4 females/dose	24 months	0%, 1.7% (1300 mg/kg bw) and 3.4% (2600 mg/kg bw) diethylene glycol (degree of purity unknown) in the diet	-	1300 mg/kg bw	Three out of 20 rats treated with 1300 mg/kg bw or 2600 mg/kg bw had bladder stones. Analysis suggested calcium oxalate stones. The rats displayed slight kidney damage (atrophy of the tubules, infiltration of lymphocytes, fibrosis) and changes in the liver (diffuse and centrilobular atrophy, fatty degeneration, bile-duct proliferation). Remark: Assignment of the observed changes to the different animals and test groups is not given, therefore the possible dose dependence of the findings cannot be assessed.	Morris *et al.* 1942 cited in Hesser 1986[126], in Deutsche Forschungsgemeinschaft (DFG) 1995[67] and in Berufsgenossenschaft der chemischen Industrie 1990[69]

Rats (Osborne-Mendel) / n=12 males/dose	2 years	1%, 2% or 4% diethylene glycol in the diet (750, 1500 and 3000 mg/kg bw/day) (study report provides no details on the purity of the diethylene glycol sample)	-	750 mg/kg bw day	At the highest dose level: - survival and growth rates reduced - mortality slightly increased. Dose-related effects on kidneys and liver by microscopy. At high doses: - hydropic degeneration and focal necrosis in the liver; - focal tubular atrophy, hyaline cast formation, hydropic degeneration, calcification and glomerular atrophy in the kidneys. At medium and low doses: less pronounced and dose-dependent. Bladder stones (calcium oxalate concretions) were found in 11, 7, and 2 animals at the high, intermediate, and low dose. Bladder tumours in 6/12 at the medium; 5/12 at the high dose group; bladder stones in all but one case. Tumours were generally benign papillomas; some had varying degrees of malignancy; one being distinctly malignant; tumour development was suggested to be due to chronic irritation by the stones. This study has only limited value since the number of animals was low and only males were used.	Fitzhugh and Nelson 1946[83] and Nelson *et al.* 1945 cited in Criteria group for occupational standards 1993[18], in Berufsgenossenschaft der chemischen Industrie 1990[69] and in Nordic steering group for assessment of health effects of chemicals 1998[17]
Rats (Carworth-Farm-Nelson) of different ages (weanling, 2 months, 1 year) / n=15-20/sex/dose	2 years (weanlings received their diets for 90 days or for a maximum of two years; two-month and one year received their diets until death or for a maximum of two years)	0%, 2 or 4% diethylene glycol (diethylene glycol containing 0.03% ethylene glycol) in food (0, 1500 and 3000 mg/kg bw/day); the levels given to the 1 year rats were adjusted during the first 6 months of the study in order to provide	-	1500 mg/kg bw/day	None of the male yearlings survived one year of treatment. About half of the male rats at 3000 mg/kg bw/day developed bladder stones (sex-related effect). Stone formation was highest (8 in 20 rats) at the 3000 mg/kg bw/day dose level. In this high-dose group, stones appeared three months sooner in yearlings than in the weanling or two-month age groups. The weanling rats fed for 90 days produced no stones. Also, no stones were found in the rats fed the 1500 mg/kg bw/day level.	Weil *et al.* 1965[84] and Weil *et al.* 1967[85]

		them with the same dose level as the youngest rats. After the first 6 months, the males at the high dose were receiving about 2-3 g/kg/day	-		The only bladder tumour was found in a high-dose male. The urinary volume of the diethylene glycol rats was almost twice that of the controls; urine acidity was increased. In this study, calcium oxalate stone implant, glass bead implant or sham operation similarly produced stones and tumours in rats that never received diethylene glycol.	
Rats (Fisher 344) / n=50/sex/group	108 weeks	0%, 1.25% and 2.5% diethylene glycol (purity 97%, ethylene glycol content was not stated) in drinking water (0, 1210 and 2630 mg/kg/day for males and 0, 1160 and 2550 mg/kg/day for females) (0, 1200 and 2600 mg/kg bw/day)	1200 mg/kg bw/day	2600 mg/kg bw/day	Increased drinking water consumption (females 17% and males 25%) and 19/50 males died compared to 13/50 in the control group; incidences of tumours and non-neoplastic lesions did not significantly differ between the three groups. Serum lactate dehydrogenase activity was increased and serum urea nitrogen was decreased in males; the creatinine phosphokinase activity and the absolute and relative weights of the lung were increased in both sexes (all of these changes were significantly changed). No urinary changes and no bladder stones, but it was not mentioned if oxalate was measured; one kidney carcinoma (1/100) and one nephroblast at the lower dose. Thus, no evidence of carcinogenic effects were found.	Hiasa *et al.* 1990[19]
Dermal						
Mice (NMRI) / n=100 females	106 weeks; s.c. injection	3, 10 and 30 mg (0.15, 0.50 and 1.5 g/kg bw/day) diethylene glycol (purity not stated) in tricaprylin once weekly	1.5 g/kg bw/day	-	No tumours in the region of the injection site (the extent of the microscopic examination was not clearly defined) or systemically; no data on non-neoplastic lesions were presented.	Dunkelberg 1987[25]

[a] NOAEL = No Observed Adverse Effect Level
[b] LOAEL = Lowest Observed Adverse Effect Level

Table E5 Genotoxicity of diethylene glycol *in vitro.*

Test system	Dose / concentration	End point	Result	Reference
(modified) Ames test with Salmonella typhimurium strains TA98 – 100 – 1535 – 1537	concentrations between 5-300 µmol/plate	Gene mutations	- (without metabolic activation)	Pfeiffer and Dunkelberg 1980[86]
Ames test with Salmonella typhimurium strains TA98 – 100 – 1535 – 1537	0, 100, 333, 1000, 3300 and 10000 µg/plate (in water)	Gene mutations	- (without metabolic activation) - (with metabolic activation)	Zeiger *et al.* 1987[87]
Ames test with Salmonella typhimurium strains TA97a – 98 – 100 – 102	0.01-100 mg	Gene mutations	- (without metabolic activation) - (with metabolic activation)	Yoshida *et al.* 1986[88] (only tabulated data in English)
Ames test with Salmonella typhimurium	up to 100 mg/ml	Gene mutations	- (without metabolic activation) - (with metabolic activation)	Slesinski *et al.* 1986[89]
Ames test with Salmonella typhimurium	not reported	Gene mutations	- (without metabolic activation) - (with metabolic activation)	NTP 1982 cited in IUCLID dataset 2000[2]
Ames test with Salmonella typhimurium strains TA98 – 100 – 102 – 104	not reported	Gene mutations	A weak mutagenic effect could be detected in strain TA104 in the presence of metabolic activation (maximum: 2.2 fold increase over the spontaneous reversion frequency at 315 µmol diethylene glycol/plate)	Krug *et al.* 1986[90]
Gene conversion test with Saccharomyces cerevisiae strain D7	not reported	Gene mutations	No gene conversion or mitotic crossing over or reverse mutation induced effects	Krug *et al.* 1986[90]
Aneuploide test with Saccharomyces cerevisiae D61M	not reported	Gene mutations	In the absence of metabolic activation, an increase of the mitotic aneuploidy rate was observed	Krug *et al.* 1986[90]
Reverse mutation test with Saccharomyces cerevisiae strain D7 and Saccharomyces cerevisiae D61M	150 and 750 µmol/ml	Gene mutations	- (not specified whether metabolic activation was used)	Krug *et al.* 1986[91]
Chromosome aberration test with Chinese hamster ovary cells	up to 50 mg/ml	Chromosome aberrations	- (without metabolic activation) - (with metabolic activation)	Slesinski *et al.* 1986[89]
HPRT-test with Chinese hamster ovary cells	up to 50 mg/ml		- (without metabolic activation) - (with metabolic activation)	Slesinski *et al.* 1986[89]
SCE-test with Chinese hamster ovary cells	up to 50 mg/ml		- (without metabolic activation) - (with metabolic activation)	Slesinski *et al.* 1986[89]
SOS chromotest with Escherichia Coli PQ37	not reported	*sfiA* gene inducing DNA damage	- (without metabolic activation) - (with metabolic activation)	Hude *et al.* 1988[92]

Table E6 Genotoxicity of diethylene glycol *in vivo.*

Test system	Dose / concentration	End point	Result	Reference
Eukaryotic Saccharomyces cerevisiae strain D7	Not reported	Gene mutations	No gene conversion or mitotic crossing over or reverse mutation induced effects	Krug *et al.* 1986[90]
Micronucleus test (species not reported)	Single i.p. injection of 60% of the LD_{50}	Chromosome aberrations	After a dose of 60% of the LD_{50}, which causes organ damage such as tubular necrosis, induction of chromosomal fragments in the micro-nucleus test is reported. This induction is suppressed when the animals are pretreated during 7 days with a low daily dose of diethylene glycol (4% of the LD_{50})	Krug *et al.* 1986[91]
Chromosome aberration test with hamsters	Exposure was by i.p. injection, by oral dose, exposure via drinking water or via the diet. I.p. injection and oral dosing included treatment times of 6, 24 or 48 h at doses between 312.5 and 7500 mg/kg bw. Although not reported, it is assumed that the data refers to single dosing and the oral dosing occurred by stomach tube. Exposure via the drinking water was carried out at levels between 0.5% and 2.0% for 1, 2 or 3 weeks. Exposure via the diet occurred for a period up to 12 weeks at dose levels between 1.25% and 5%.	Chromosome aberrations	A number of 100 cells was analysed for all concentrations in the various exposure scenarios. The background in the controls was one or two aberrations in 100 analysed cells for all scenarios, regardless the treatment time. Slight increases in the number of chromosome aberrations were observed. After 6, 24 and 48 hr, slight increases in the incidence of chromosome aberrations were found after single i.p. injection of 1250 mg/kg (3, 6 and 3 aberrations); 2500 mg/kg (4, 7 and 2 aberrations) and 5000 mg/kg (7, 5 and 7 aberrations), respectively (7500 mg/kg was not done). Oral dosing induced chromosome aberrations only at 7500 mg/kg; 4, 3 and 4 aberrations after 6, 24 and 48 hr, respectively. Exposure via the drinking water resulted in an increase of aberrations at all dose levels. At the lowest level the highest (0.5%) number of aberrations (4) was observed after 2 weeks of exposure. At 1.0 and 2.0% the most pronounced effects were 4 aberrations (after 2 weeks) and 6 aberrations (after 1 week), respectively. After dietary exposure for 12 weeks at dose levels of 1.25-5%, the number of chromosome aberrations was similar to controls (1-2 aberrations per 100 cells).	Yoshida *et al.* 1986[88] (only tabulated data in English)
Hamsters	1/5 of LD_{50} by gavage	Chromosome aberrations	Increase in chromosome damage in the bone marrow cells	Barilyak 1985 (article in Russian) cited in BIBRA 1993[48] and in IUCLID dataset 2000[2]
Dominant lethal assay with rats	Not reported		Dominant lethal mutations in spermatides	Barilyak 1985 (article in Russian) cited in IUCLID dataset 2000[2]

Table E7 Animal carcinogenicity studies of diethylene glycol.

Species/Strain/No. per Sex per Group	Exposure duration	Concentration/dose tested	NOAEL[1]	LOAEL[2]	(Critical) effects	Reference
Inhalation						
Mice / n=16	2 hour/day; 6-7 months	Damp aerosol mist at 30-35 °C; 4-5 mg/m³ (ca. 0.92 ml/m³) diethylene glycol (purity not stated)	-	4-5 mg/m³	Study is described in Russian. In reviews, the following effects are mentioned: bronchitis, pneumonitis, liver (slight protein dystrophy) and kidney damage (dystrophy of the epithelium and round-cell infiltrations); 10 of 16 animals developed tumours 2.5 to 11 months after the experiment; one mouse had a lymphosarcoma in the back of the neck; another had a smooth-cell, non-keratinizing tumour of the mammary gland; 7 mice had adenocarcinomas of the mammary glands; one animal had a solid tumour (not further defined). Reviews state that this study is of limited value: - no information on the manner of determination of the concentration and analysis of the aerosol-vapour mixture was provided; - the treated animals were maintained at elevated temperatures but it is uncertain whether the control animals were under a similar heat stress; - the incidence of tumours in the 20 untreated controls was not recorded.	Sanina 1968[128] cited in BIBRA 1993[48], in Cavender and Sowinski 1994[125], in Deutsche Forschungsgemeinschaft (DFG) 1995[67] and in Berufsgenossenschaft der chemischen Industrie 1990[69]
Oral						
Rats (Osborne-Mendel) / n=12 males/dose	2 years	1%, 2% or 4% diethylene glycol in the diet (750, 1500 and 3000 mg/kg bw/day) (study report provides no details on the purity of the diethylene glycol sample)	-	750 mg/kg bw day	At highest dose level: reduced survival and growth rates; mortality slightly increased; dose-related effects on kidneys and liver including hydropic degeneration, focal tubular atrophy, hyaline cast formation, calcification and glomerular atrophy; focal necrosis in the liver. At medium and low dose level: less pronounced dose-dependent changes.	Fitzhugh and Nelson 1946[83] and Nelson *et al.* 1945 and cited in Criteria group for occupational standards 1993[18], in Berufsgenossenschaft der chemischen Industrie

					Bladder stones (calcium oxalate concretions) in 11, 7 and 2 animals at the high, intermediate and low dose; bladder tumours in 6/12 of the animals at the medium and 5/12 of the animals at the high dose level; in all but one case (not stated in which group), bladder stones were also detected. The tumours were generally benign papillomas, some showing varying degrees of malignancy, and one being distinctly malignant. The investigators suggested chronic irritation by the stones as the cause. This study has limited value as the number of animals was low and only males were used.	1990[69] and in Nordic steering group for assessment of health effects of chemicals 1998[17]
Rats (Carworth-Farm-Nelson) of different ages (weanling, 2 months, 1 year) / n=15-20/sex/dose	2 years (weanlings received their diets for 90 days or for a maximum of two years; two-month and one year received their diets until death or for a maximum of two years)	0%, 2 or 4% diethylene glycol (diethylene glycol containing 0.03% ethylene glycol) in food (0, 1500 and 3000 mg/kg bw/day); the levels given to the 1 year rats were adjusted during the first 6 months of the study in order to provide them with the same dose level as the youngest rats. After the first 6 months, the males at the high dose were receiving about 2-3 g/kg/day	-	1500 mg/kg bw/day	None of the male yearlings survived one year of treatment. About half of the male rats at 3000 mg/kg bw/day developed bladder stones (sex-related effect). The highest stone formation was 8 in 20 rats at the 3000 mg/kg bw/day dosage level. In this high-dose group, stones appeared three months sooner in yearlings than in the weanling or two-month age groups. The weanling rats fed for 90 days produced no stones. Also, no stones were found in the rats fed the 1500 mg/kg bw/day level. The only bladder tumour was found in a high-dose male (weanling which died after 362 days) (probably due to mechanical irritation). The urinary volume of the diethylene glycol rats was almost twice that of the controls while urinary acidity was significantly increased but to a lesser extent. In this study, calcium oxalate stone implant, glass bead implant or sham operation resulted in similar production of stones and of tumours as well in the rats that never received diethylene glycol.	Weil *et al.* 1965[84] and Weil *et al.* 1967[85]

Rats (Fisher 344) / n=20 males	32 weeks	2% diethylene glycol (purity not stated) in the diet (20000 mg/kg diet) with or without 0.05% N-butyl-N-(4-hydroxybutyl)nitrosamine (as an initiator for bladder tumours)	2%	-	A slight increase in crystal formation in the urinary bladder was observed in diethylene glycol-treated rats but the incidence of bladder tumours was not significantly different as compared with controls. From the absence of a promoting effect it was considered that formation of urinary crystals plays a very limited role, if any, in urinary bladder carcinogenesis	Masui *et al.* 1988[94]
Rats	Initial single dose: 200 mg/kg of diethylnitrosamine (DEN) i.p.; after 2 weeks followed by diethylene glycol for 6 weeks; all rats had partial hepatectomy at week 3; necropsy after week 6.	10 g/kg diethylene glycol (no information available with regard to diethylene glycol purity) in food	10 g/kg diethylene glycol in food	-	Carcinogenic potential was scored by comparing the number and area per cm^2 of induced glutathione S-transferase placental form-positive (GST-P+) foci in the liver with those of the corresponding control group given DEN alone. Positive was scored for a significant increase in the value of GST-P+ foci, negative for no change or a decrease. DEN in combination with diethylene glycol did not increase S-transferase placental form-positive (GST-P+) foci in the liver.	Ito *et al.* 1988[95]
Rats (Fisher 344) / n=50/sex/group	108 weeks	0%, 1.25% and 2.5% diethylene glycol (diethylene glycol purity 97%, ethylene glycol content was not stated) in drinking water (0, 1210 and 2630 mg/kg/day for males and 0, 1160 and 2550 mg/kg bw/day for females) (0, 1200 and 2600 mg/kg bw/day)	2600 mg/kg bw/day	-	Only one kidney carcinoma was detected (1/100). At the lower dose one kidney carcinoma and one nephroblast were recorded. Thus, no evidence of carcinogenic effects were observed.	Hiasa *et al.* 1990[19]

Rats (Fisher 344) / n=20 males	30 weeks	2.5% diethylene glycol (diethylene glycol purity 97%, ethylene glycol content was not stated) in drinking water (2600 mg/kg bw/day)	2600 mg/kg bw/day	-	No renal promoting potential was evident after initiation with N-ethyl-N-hydroxyethylnitrosamine for two weeks.	Hiasa *et al.* 1990[19]
Rats	2 days	In a carcinogenicity study, 5% diethylene glycol (purity not stated) in drinking water (5200 mg/kg bw/day) was used as a negative control	-	5200 mg/kg bw/day	Since treated animals died of nephrosis caused by 5% diethylene glycol in drinking water for more than 3 days in this study, exposure was limited to 2 days. As a result, numbers of adenomatous hyperplasia induced by diethylene glycol did not increase significantly. Diethylene glycol showed a significant but small promoting effect. Diethylene glycol may have weak promoting activity.	Hiasa *et al.* 1991[96]
Dermal						
Mice	3 times weekly (diethylene glycol was added to cigarette tobacco as a humectant and applied on the dorsal skin)	2% diethylene glycol (no information available with regard to diethylene glycol purity) (0.8 g/dose) (controls were administered smoke condensate without addition of diethylene glycol)	2% diethylene glycol (0.8 g/dose)	-	The incidence of skin tumours was not significantly different from the controls.	Dontenwill *et al.* 1970[97]
Mice / n=74	2 years; three times a week	two drops of undiluted diethylene glycol (no information available with regard to diethylene glycol purity) on the uncovered skin of the back (3 g/kg bw per application)	two drops of undiluted diethylene glycol on the skin (3 g/kg bw / application	-	No evidence of skin carcinogenicity (only one animal developed an papiloma)	Vasil'eva *et al.* 1971 cited in BIBRA 1993[48]

Mice (NMRI) / n=100 females	106 weeks; s.c. injection	3, 10 and 30 mg (0.15, 0.50 and 1.5 g/kg bw) diethylene glycol (no information available on diethylene glycol purity) in tricaprylin once weekly	1.5 g/kg	-	No tumours in the region of the injection site (the extent of the microscopic examination was not clearly defined) or systemically; no data on non-neoplastic lesions were presented.	Dunkelberg 1987[25]

Table E8 Animal reproduction toxicity studies of diethylene glycol.

Species/Strain/No. per Sex per Group	Exposure duration	Concentration/dose tested	NOAEL[a]	LOAEL[b]	Critical effect	Reference
Fertility						
Inhalation						
Rats / n=10 females/dose	4 hour daily throughout pregnancy	10.5, 46 and 328 mg/m^3	46 mg/m^3	328 mg/m^3	Remark: Study not obtainable. Reduced number of viable animals (limited Russian report)	Barilyak 1989 cited in BIBRA 1993[48]
Oral						
Mice (Swiss CD-1) / n=20 pairs/dose)	Two-generation study employing a continuous breeding protocol; 4 months	0, 0.35, 1.75 and 3.5% diethylene glycol (purity >99%) in drinking water (equivalent to 0, 610, 3100 and 6100 mg/kg bw/day)	3100 mg/kg bw/day	6100 mg/kg bw/day	F0 body weight unchanged in task 2 mating period; litters/pair decreased by 12%; live pups/litter decreased by 32%; and pup weight adjusted for litter size was reduced by 12% at top dose; significant increase in cumulative days to litter; significant decrease in the number of pairs producing the third, fourth, and fifth litters (increase in the number of infertile pairs) at top dose; In crossover mating: number of pups/litter was equivalent across the three groups, but adjusted pup weight was reduced by 10% in the control male x 6100 mg/kg bw/day female mating.	NTP 1984[98]; Williams *et al.* 1990[20]; Anonymous1997[130] and Morrissey *et al.* 1989[131] Bates *et al.* 1991[99] Nordic steering group for assessment of health effects of chemicals, 1998[17]

					At 6100 mg/kg bw/day: no treatment-related changes in male organ weights or histopathology; female body weight was reduced by 7%; adjusted organ weights were unchanged. For F_1 mating trial with 3100 mg/kg bw/day group (insufficient mice available from top dose): pup survival to mating not affected at pnd 74; no treatment-related changes in number or weight of F_2 pups in Task 4 mating trial. After birth of F2 and estrous evaluation of F1, the F_1 mice were killed for necropsy: 11% (males) and 7% (females) decrease in body weight; organ weights and sperm indices not affected. Summary: diethylene glycol at 6100 mg/kg bw/day was a reproductive toxicant in Swiss mice, based on reductions in litters/pair and in mean litter size, with slight maternal F0 toxicity (7% decrease of bw) and decreased F1 bw at birth and poor postnatal survival; at 3100 mg/kg bw/day: decreased bw of both sexes at weaning, at onset of mating, and at necropsy; no adverse effects on reproduction. Some offspring (at > 6 g/kg bw/day) were grossly abnormal: craniofacial malformations, embryotoxicity, decreased postnatal survival; fifth or final litters consisted of fewer live pups with significantly reduced birth weights; 12% of liveborn pups and 95% of pups dead on postnatal day 0 had craniofacial malformations including exencephaly and cleft palate; at postnatal day 2, 50% of malformed pups had died.	
Rats (female treated rats were mated with untreated males); rats (male and female rats were housed together)	not reported not reported	0.15 and 0.3 g/kg bw/day in drinking water; 0.5% diethylene glycol (0.3 g/kg bw/day) in drinking water	- -	0.15 g/kg bw/day 0.5% diethylene glycol (0.3 g/kg bw/day)	Reduced fertility No pregnancies resulted	Holck 1937 cited in BIBRA 1993[48]

Rats / n=10/sex/dose	Two-generation study (12 weeks)	1.0 ml/100 g bw of a 20% aqueous solution of diethylene glycol ('pure' without further specification) (2.2 g/kg bw/day)	1.0 ml/100 g bw of a 20% aqueous solution of diethylene glycol (2.2 g/kg bw/day)	-	No impairment of reproduction (fertility) and no embryotoxic effects were observed	Wegener 1953 cited in Hellwig *et al.* 1995[23] and in BIBRA, 1993[48]
Rats (SD) / n=30/sex/dose	73 days prior to pairing and up to the end of the study	0, 150, 500 and 1500 mg/kg bw/day diethylene glycol (purity not stated) in drinking water	1500 mg/kg bw/day	-	1500 mg/kg/d: Increase of the relative kidney weight in male rats of the P and of the F1 generation. Nevertheless, neither reproduction of the P generation nor prenatal development, survival, and growth of the F1 generation were adversely affected	Rodwell *et al.* 1987[132]
Rats	2 years	2 and 3.4% diethylene glycol (no information available with regard to purity) in the diet (1 and 1.71 g/kg bw/day)	3.42% (1.71 g/kg bw/day)	-	No observable changes in testicular histology; no other aspects of male reproductive function were analysed in this study	Morris *et al.* 1942 cited in Williams *et al.* 1990[20]
Other routes						
Rabbits / n=2	7 days; injection	2.23 g/day/animal (no information available with regard to purity of diethylene glycol)	-	2.23 g/day /animal	Degeneration of germinal epithelium in the testes (old study: 1938)	Wiley *et al.* 1938 cited in Hardin 1983[133]
Developmental toxicity						
Inhalation						
Rabbits (Dutch) / n=8/dose	Gestational days 6-18	0, 100, 250 and 450 ppm diethylene glycol (equivalent to 0, 440, 1100 and 1980 mg/m^3) (no information available with regard to purity of diethylene glycol)	-	440 mg/m^3	Significantly decreased body weight and food consumption (1980 mg/m^3); increased mean % pre-implantation losses and litters with any pre-implantation loss (440 and 1980 mg/m^3); mean number of intra-uterine deaths (1980 mg/m^3); decreased mean foetal and gravid uterine weights (at all doses). No significant differences in the following: maternal mortality, haematology values, organ weights, macroscopic observations, post-implantation loss, and mean number of live foetuses. One high-dose group rabbit had marked ataxia, loss of withdrawal reflex, slight head tremors, and (at necropsy) 100% post-implantation losses.	cited in HSDB, 2003[40]
Oral						

Mice (CD-1 Swiss) / n=26-31/dose	Days 6-15 of gestation	0, 1250, 5000 and 10000 mg/kg bw/day diethylene glycol (purity not stated) in drinking water	1250 mg/kg bw/day (adult) 5000 mg/kg bw/day (developmen-tal)	5000 mg/kg bw/day (adult) 10000 mg/kg bw/day (developmen-tal)	Maternal body weights not significantly different at any dose. At 5,000 and 10,000 mg/kg bw/day: relative water intake significantly increased over control for every interval starting at GD 6; necropsy on GD 17 showed significantly increased absolute (g) and relative (% body weight) kidney weights. At 10,000 mg/kg bw/day: maternal animals showed significantly decreased relative (g/kg body weight/day) food consumption from GD 6 to 12; renal tubular degeneration was found in 3/28 of the pregnant females versus 0/20 of pregnant control females. No effects of diethylene glycol were observed on pre- or post-implantation loss. The mean foetal body weight on GD 17 showed a significant decreasing trend (99%, 96%, and 85% of control from low to high dose); mean foetal body weight was significantly decreased (0.865 g in the high dose group vs 1.012 g in control group). No significant external, visceral and skeletal malformations at any dose level. The decrease in foetal body weight indicated developmental toxicity at the 10,000 mg/kg bw/day dose level. Summary: no maternal or developmental toxicity at 1250 mg/kg bw/day; significant maternal toxicity at 5000 mg/kg bw/day (no evidence of developmental toxicity); maternal toxicity and developmental toxicity at 10,000 mg/kg bw/day (death in 1/28 pregnant dams).	NTP 1991[100] and Bates *et al.* 1991[99]
Mice (CD-1) / n=50 plug-positive females/dose	Gestational days 7-14	0 and 10 ml/kg diethylene glycol (purity: 97%, no further specification) (equivalent to 11.8 g/kg bw/day) by gavage	11.8 g/kg bw/day	-	2/50 (4%) of the dams died; pup weight gain after 3 days was the only variable significantly ($p<0.05$) altered from control (0.7 g in the control group and 0.6 g in the diethylene glycol-group).	Schuler *et al.* 1984[134] and Hardin *et al.* 1987[135]

Rats (pregnant) / n-14/dose	Gestational days 0-20	0, 0.2, 1 and 5% diethylene glycol in food (no information available with regard to purity) (equivalent to 0, 140, 684 and 3556 mg/kg bw/day)	3556 mg/kg bw/day	-	No significant differences in the prevalence of foetal lethality or in the prevalence of malformations. The postnatal viability of the offspring of dams fed diethylene glycol was not significantly altered from control.	Kawasaki *et al.* 1984 cited in NTP 1991[100]
Rats	Gestational days 0-20	not reported	-	50000 mg/kg in the diet (3300 mg/kg bw)	Slight reduction in the weight of the neonates; musculosketal abnormalities	Kawasaki *et al.* 1984, Shephard 1989 and Lewis 1991 cited in Nordic steering group for assessment of health effects of chemicals 1998[17]
Rats / n=25/dose	Gestational days 6-15	0, 1.0, 4.0 and 8.0 ml/kg bw/day by gavage (0, 1115, 4460 and 8920 mg/kg bw/day diethylene glycol (purity not stated)	1.0 ml/kg bw /day (1120 mg/kg bw/day)	4.0 ml/kg bw/day (4460 mg/kg bw/day) (in combination with maternal toxicity)	At 4460 and 8920 mg/kg bw/day: developmental delay only in the presence of maternal effects. At 8920 mg/kg bw/day: 3 dams died on gestation day 11; maternal effects in surviving dams included reduced gestational body weight, food consumption, increased water consumption and kidney weights. Maternal kidneys showed interstitial nephritis and tubular basophilia indicative of renal tubule damage and repair. At 4460 mg/kg bw/day: increased water consumption, reduced food consumption, decreased corrected weight gain. At 8920 mg/kg bw/day: reduced litter weights; increased incidence of five skeletal variations in foetuses. At 4460 mg/kg bw/day: one skeletal variation increased. Thus, minor developmental effects, developmental delay, reduced litter weight and (an) increase(s) in incidence(s) of skeletal variation(s) were seen in the presence of maternal effects from 4460 mg/kg bw/day. No foetal malformations were observed at any dose level.	Neeper-Bradley *et al.* 1992[101]
Rats (Wistar)	Gestational days 6-15	200, 100, 5000 mg/kg bw in distilled water by gavage	5000 mg/kg bw/day	-	NOAEL for maternal toxicity and embryotoxicity/teratogenicity	RCC 1985 cited in IUCLID dataset 2000[2]

Rats	Gestational days 6-15	38210 and 76420 mg/kg bw (no information available with regard to purity)	-	38210 mg/kg bw	Offspring displayed musculoskeletal abnormalities; at a dose of 76420 mg/kg fetotoxicity was observed	Reference USEPA 1984 cited in IUCLID dataset 2000[2]
Rats	Multigeneration study	343 g/kg bw (no information available with regard to purity)	-	343 g/kg	Female mice displayed and altered sex ratio and foetal death in offspring	Reference USEPA cited in IUCLID dataset, 2000[2]
Rabbits (pregnant) / n=5	Day 7 postinsemination – day 19 postinsemination	0, 400 and 1000 mg/kg bw/day diethylene glycol (purity not stated) by gavage	-	1000 mg/kg bw (maternal effects)	A marginal impairment of the body weight gain of the dams was the only possible compound-related finding (maternal toxicity)	BASF AG 1987 cited in Hellwig *et al.* 1995[23]
Rabbits (pregnant Himalayan) / n=15	Day 7 postinsemination – day 19 postinsemination	0, 100, 400 and 1000 mg/kg bw diethylene glycol (purity 98.8%) (by gavage) (15 female rabbits per group)	> 1000 mg/kg bw	-	No signs of maternal toxicity or embryo-/fetotoxicity were observed	Hellwig *et al.* 1995[23]
Other routes						
Hamsters (pregnant Syrian hamster)	One intraperitoneal injection on day 8 of pregnancy	2.25 to 4 ml/kg bw diethylene glycol (purity not stated) (2.51, 2.80, 3.08, 3.36, 3.92 and 4.46 g/kg bw)	-	2.51 g/kg	Foetuses from treated animals examined at autopsy on day 15: decreased body weights; neural-tube effects (exencephaly, cranial bleb and myelomeningocele); dose-related deficit in the number of live, externally non-malformed foetuses and dose-related increase in the number of live externally abnormal foetuses; few dead full-term foetuses, but dose-related increase in late resorptions; possibly an indirect result of maternal toxicity or an effect of diethylene glycol (or metabolites) on conception. Dams receiving 4.48 and 3.92 g/kg bw died; survival in lower dosage groups was dose dependent ;reductions in maternal weight gain occurred at all doses.	Renwick and Cameron, 1992[24]

[a] NOAEL = No Observed Adverse Effect Level

[b] LOAEL = Lowest Observed Adverse Effect Level